ATOM TRANSFER REACTIONS OF AMINO ACID DERIVATIVES

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M. P. Hay

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ABSTRACT.

Investigations of atom transfer reactions of amino acid derivatives are described in this thesis. Reaction of N-benzoylvaline methyl ester (29a) with sulphuryl chloride gave the β-chlorovaline derivative (30a) and lesser amounts of diastereoisomers of the γ-chlorovaline derivative (31a). Studies of reactions of the valine derivatives (29a-c) with sulphuryl chloride, and of the photolyses of the corresponding N-chlorovaline derivatives (39a-c) have shown that the reaction of (29a) with sulphuryl chloride and the photolysis of (39a) involve regioselective intermolecular transfer of the respective β-valinyl hydrogens. There is no evidence of reaction at the corresponding α-positions. These reactions establish the chemical validity of a regiospecific hydrogen atom transfer proposed in penicillin biosynthesis and in the β-hydroxylation of valine residues in peptides.

Factors affecting the production of amidocarboxy-substituted radicals have been investigated by examining reactions of derivatives of valine, alanine and glycine with a number of reagents. The variation in the regioselectivity of reactions of these compounds is typified by the reactions of (29a) with sulphuryl chloride and N-bromosuccinimide. Whereas the reaction of (29a) with sulphuryl chloride involves hydrogen atom abstraction from the β-position of (29a) with subsequent chlorine atom incorporation to give (30a), the reaction with N-bromosuccinimide proceeds *via* hydrogen atom abstraction from the α-position of (29a) and yields the dibromovaline derivative (76). The studies indicate that amidocarboxy-substituted radicals such as (34a) are considerably more stable than, for example, the tertiary alkyl radical (32a), but hydrogen atom transfer reactions may afford the less stable products if electrophilic radicals are involved in the hydrogen atom abstraction and if there is little development of radical character in the transition state of the reaction.

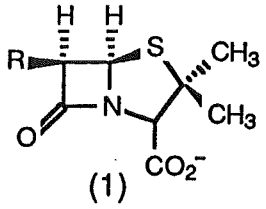
The preferential reactivity of glycine residues in free radical reactions of proteins, peptides and other amino acid derivatives has been investigated by

examination of reactions of derivatives of valine, alanine, glycine, methyl pyroglutamate and proline with NBS. The previously unexplained reactivity of glycine derivatives has been rationalised in terms of steric interactions and their effect on the conformation of the amidocarboxy-substituted captodative radical (41).

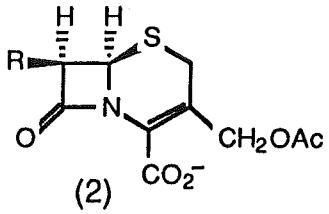
The selective reactivity of glycine derivatives has been exploited synthetically. Reaction of the α-bromoglycine derivative (112a), prepared by reaction of the glycine derivative (93a) with NBS, with hexabutylditin under strictly anhydrous conditions gave the diasteroisomers of the glycine dimer (131). Otherwise the major products were the ethers (132a) and (132b). Selective functionalisation of the dipeptide (126a) at the α-glycyl position was achieved by reaction with NBS. The regioselectively labelled dipeptide (126b) was synthesised by reaction of the bromide (127) with triphenyltin deuteride.

INTRODUCTION.

Investigations of the biosynthesis of β-lactam antibiotics such as the penicillins (1) and cephalosporins (2) have continued for the last forty years.[1] The penicillins (1) possess a common bicyclic ring system consisting of a β-lactam ring *cis*-fused to a thiazolidine ring. Different penicillins vary in the nature of the N-acyl side chain, which may be altered biosynthetically by changing the side chain precursor in the fermentation.[2] In the absence of a suitable side chain precursor, fermentation of *Penicillin chrysogenum* leads to the formation of 6-aminopenicillanic acid (1a) and isopenicillin N (1b).[3,4] The former compound is



(1)



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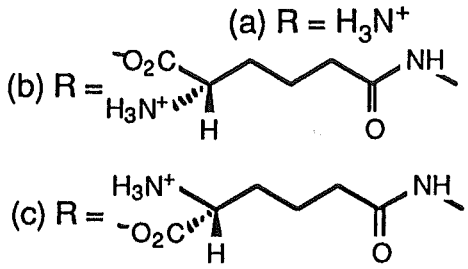
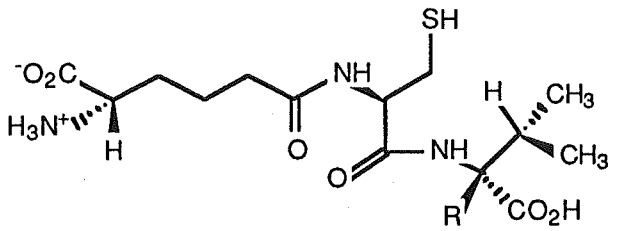


an important intermediate in the synthesis of semi-synthetic penicillins, produced by acylation of the free amino group, while much of the investigation of the biosynthesis of the β-lactams has centred on isopenicillin N (1b).

Cephalosporin C (2c) was isolated from *Cephalosporium acremonium*[5] and was shown to have a structure similar to the penicillins (1).[6] The cephalosporins (2) differ from the penicillins (1) only in that they contain the six-membered 3-cephem ring rather than the five-membered penam ring. The aminoadipoyl side chain of (2c) was found to have the R-configuration in common with penicillin N (1c) and was thus thought to be biosynthesised from a

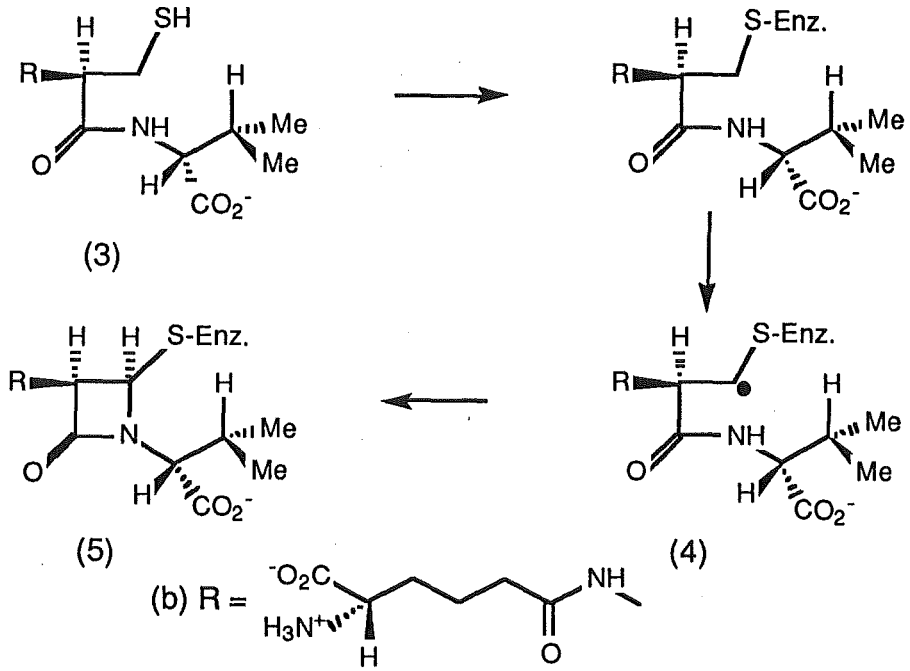
common precursor.

There have been many studies aimed at establishing the biosynthetic precursors of the penicillins (1) and cephalosporins (2). Most of the early experiments consisted of the addition of potential biosynthetic precursors to cultures and observing the effect on the yield of penicillins (1). This approach was not very satisfactory; however, with the application of isotope labelling techniques to this problem, progress was made. The isolation of the tripeptide δ–(α-aminoadipoyl)-cysteinylvaline (3a) by Arnstein *et. al.* [7] from *P. chrysogenum* led to this peptide being proposed as an intermediate in penicillin biosynthesis. The configuration of the peptide was later determined to be δ–(S-α-aminoadipoyl)-S-cysteinyl-R-valine (3a)[8] which is consistent with the configuration, at the corresponding positions, of isopenicillin N (1b) isolated from *P. chrysogenum*.

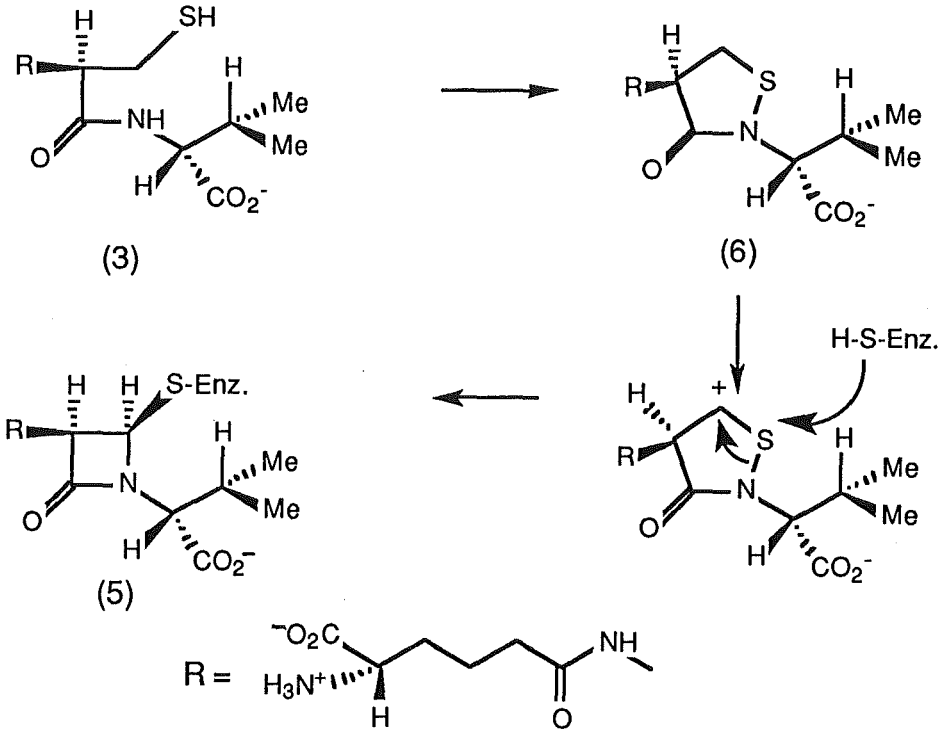


(3)
(a) R = H
(b) R = D

Recently a single enzyme has been isolated which catalyses the formation of isopenicillin N (1b) from Arnstein's tripeptide (3a).[9] The enzymatic reaction requires ferrous ions and one equivalent of oxygen.[10] The stepwise conversion of the tripeptide (3a) to isopenicillin N (1b) has been shown to involve two separate carbon-hydrogen bond breaking steps, the first of which occurs at the cysteinyl β-position and the second at the valinyl β-position.[11] The formation of an intermediate, enzyme bound β-lactam (5) has been postulated.[9,12,13,14,15] Labelling studies and related experiments[16,17,18] have shown that the ring



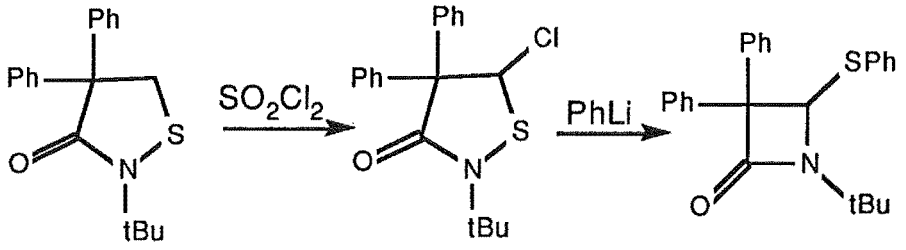
Scheme 1.



Scheme 2.

closures affording the β-lactam and thiazolidine entities occur with retention of configuration at the appropriate carbons, *i.e.* , at the β-carbon of the cysteine residue and at the β-carbon of the valine residue.

Model studies on the formation of the β-lactam ring in (1b) indicated that it may be formed by free radical processes, and a mechanism involving formation of a carbon-centred radical at the β-carbon of the cysteine residue (4), followed by direct cyclisation to give the β-lactam (5), was proposed (Scheme 1).[13,14] An alternative mechanism, proposed by Easton,[15] involved the formation of the β-lactam (5) from the isothiazolidinone (6),*via* an ionic process (Scheme 2). The proposed formation of the β-lactam (5) from the isothiazolidinone (6) is supported by the reaction of the model compound (7) with sulphuryl chloride to give (8), which reacted with phenyllithium to give the β-lactam (9) (Scheme 3).

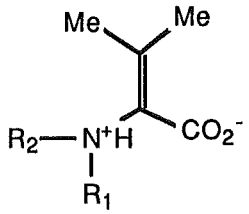


(7) (8) (9)

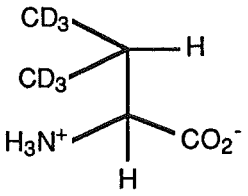
Scheme 3.

Retention of the deuterium during the incorporation of S-α-aminoadipoyl-S-cysteinyl-R-[2-^{2}H]-valine (3b) into isopenicillin N (1b) by an extract of *C. acremonium* showed that the dehydrovaline moiety (10) is not involved as an intermediate in the formation of the thiazolidine ring of (1b).[17] The incorporation of [4-$^2H_6$]-valine (11) into isopenicillin N (1b) with retention of all six deuterium atoms[17,18,19] limits the type of biomechanism possible for the formation of the carbon-sulphur bond. A free radical pathway has been proposed for the formation of the carbon-sulphur bond, involving the formation of the carbon radical (12) by hydrogen atom transfer from the valine derivative (5), with

subsequent substitution at sulphur to give isopenicillin N (1b) (Scheme 4).[20]

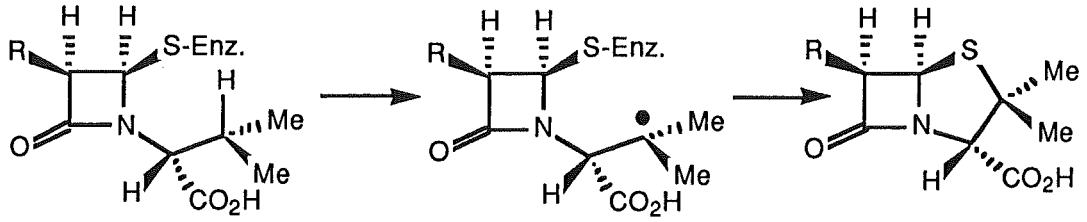


(10)

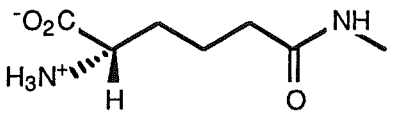


(11)

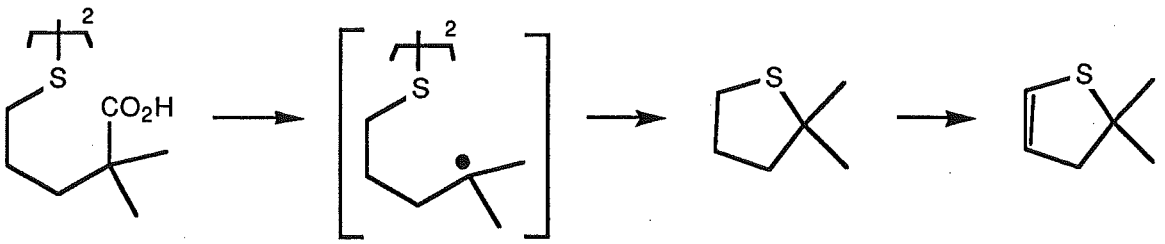
The model used originally in support of this proposal was the reaction of the disulphide (13) to form the thiophens (15) and (16) (Scheme 5). The products were attributed to reaction involving the formation of the radical (14) by oxidative decarboxylation, with subsequent cyclisation to give the thiophen (15).



(5) (12) (1b)

R = 

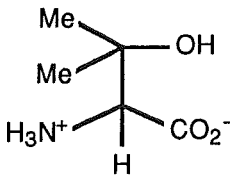
Scheme 4.



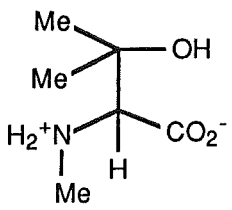
(13) (14) (15) (16)

Scheme 5.

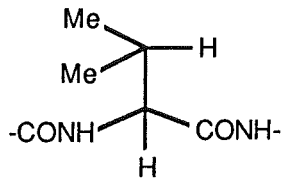
Reactions analogous to the regioselective β-hydrogen transfer from (5) to give (12) in penicillin biosynthesis may be involved in the biosynthesis of S-β-hydroxyvaline (17) and β–hydroxy-N-methylvaline (18). S-β–Hydroxyvaline (17) occurs in nature as a constituent of the tripeptide P1, isolated from *Cephalosporium sp.* [21] It is also found in the antibiotic berninamycin A[22] and in the antibiotics YA 56, X and Y,[23] isolated from *Streptomyces sp.* The related β–hydroxy-N-methylvaline (18) was found to be a constituent of the anti-tumour antibiotics BBM-928 A,B and C.[24,25] There is evidence that enzymatic hydroxylations and other biological oxidations involve free radical intermediates.[26]



(17)



(18)

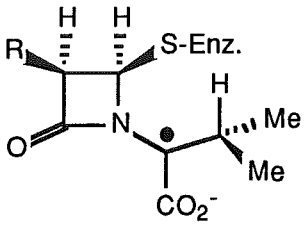


(19)

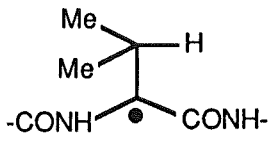
A fundamental assumption in the proposals that the conversion of the β-lactam (5) to isopenicillin N (1b) occurs as shown in Scheme 4, and that β-hydroxylation of valine derivatives is a free radical process, is that hydrogen atom transfer from valine derivatives such as (5) and (19) occurs selectively at the β-position. This point is emphasised because the α-centred radicals (20) and (21) would be expected to be thermodynamically more stable than the corresponding β-centred radicals (12) and (22). The radicals (20) and (21) are expected to be stabilised by the combined effect of the resonance electron-donating amido and electron-withdrawing carboxy substituents.

The captodative effect was postulated by Viehe *et. al.* [27] as the combined action of an electron-withdrawing (capto) and an electron-donating (dative) substituent on a radical centre, leading to enhanced stabilisation of the radical.

The captodative effect has been examined before. Balaban[28] used the term "push-pull radicals" to describe stable nitrogen radicals, the stability of which he ascribed to conjugation of the radical with an electron-donating and an electron-withdrawing substituent. Katritzky *et. al.* [29] described radicals where electron-donating and electron-withdrawing substituents interact with the same radical



(20)



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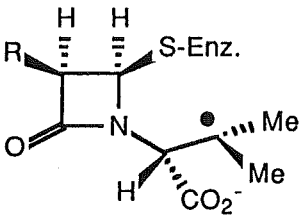
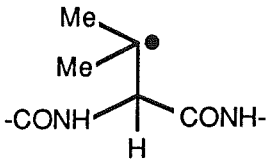


(12)

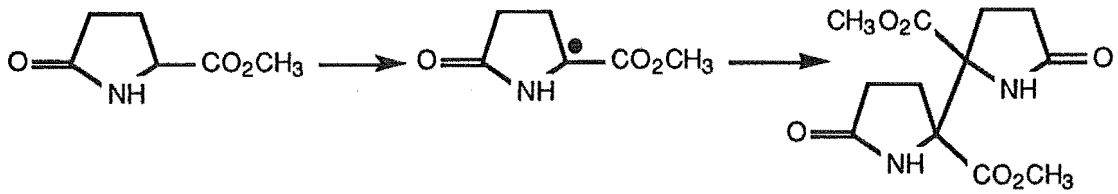


(22)

centre as "merostabilised". Although the concept has been used to explain the unexpected stability of some radicals and predict the existence of new stable nitrogen radicals[30] there has been much debate on whether the captodative effect provides a synergistic stabilisation. The proof required is that an electron-donating and an electron-withdrawing substituent, at a radical centre, provide a stabilising effect which is greater than the combined sum of the effects produced by the individual substituents. In spite of numerous attempts to prove this, the general observation is that the combined stabilising effect of a "capto" and a "dative" group is, within experimental error, equal to the sum of the individual effects.[31] Recently, evidence in favour of the captodative effect was obtained from an e.s.r. study,[32] but it is thought that the captodative effect in simple organic systems is likely to be so small that it is beyond the range of true

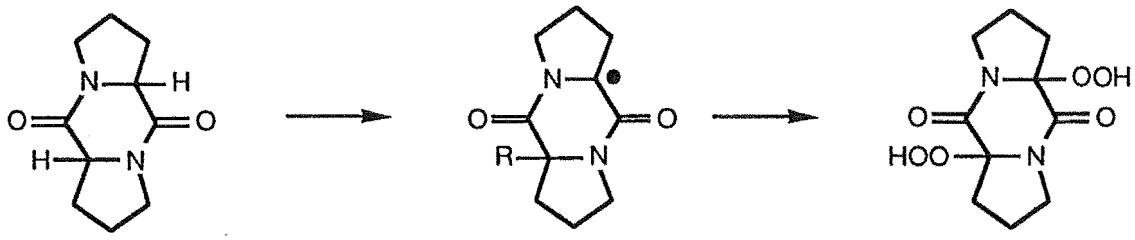
thermochemical measurement.[33]

Nevertheless, radicals such as (20) and (21) may be described as captodative radicals, stabilised by the combined, but not necessarily synergistic, action of resonance electron-donating amido and electron-withdrawing carboxy substituents. The stability of radicals of this type has been demonstrated. Irradiation of a mixture of methyl pyroglutamate (23a) and di-*t*-butyl peroxide gave a diastereomeric mixture of dimethyl di-αα'-pyroglutamate (25), presumably *via* the α-centred captodative radical (24).[34] The piperazinedione (26) reacted with oxygen forming the diperoxide (28), presumably *via* the radicals (27a) and (27b).[35] On the basis of this evidence, amidocarboxy-substituted radicals such as (20) and (21) are expected to be considerably more stable than, and to be formed in preference to, the corresponding tertiary alkyl radicals (12) and (22).



(23a) (24) (25)

Scheme 6.



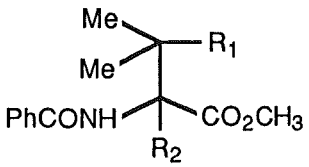
(26) (27) (28)

(a) R = H
(b) R = OOH

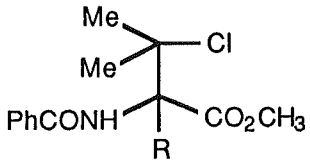
Scheme 7.

Studies to examine the anomaly inherent in the proposal of the β-centred

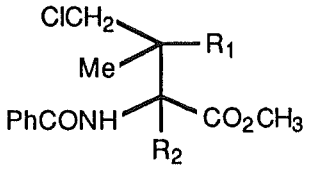
radical (12) as an intermediate in the reaction of (5) to give (1b) when formation of the more stable α-centred radical (20) is possible, were carried out by Easton and Bowman.[36] N-Benzoylvaline methyl ester (29a) was heated at reflux with sulphuryl chloride in carbon tetrachloride, with benzoyl peroxide as an initiator, to give the β-chlorovaline derivative (30a) and diastereoisomers of the γ-chlorovaline derivative (31a). Analysis of mixtures at 10-50% reaction showed a ratio of formation of the β-chlorovaline derivative (30a) to diastereoisomers of the γ-chlorovaline derivative (31a) of *ca.* 2:1:1 and no other products were detected. An increase in selectivity for β-carbon-hydrogen bond homolysis versus γ-carbon-hydrogen bond homolysis was observed when the solvent was changed from carbon tetrachloride to benzene. The selectivity of 6:1, on a per hydrogen basis, for reaction in carbon tetrachloride, attributable to the relative reactivities of tertiary and primary hydrogens,[37] increased to 9:1 with the use of benzene as the solvent.



(29)
(a) $R_1 = R_2 = H$
(b) $R_1 = D$, $R_2 = H$
(c) $R_1 = H$, $R_2 = D$



(30)
(a) R = H
(b) R = D



(31)
(a) $R_1 = R_2 = H$
(b) $R_1 = D$, $R_2 = H$
(c) $R_1 = H$, $R_2 = D$

A number of free radical chlorinations using sulphuryl chloride have been described by Brown and coworkers[38] and the mechanism is thought to involve the following propagation steps.

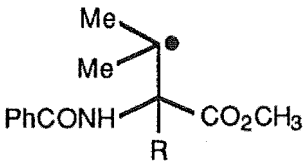
$$R^{\bullet} + SO_2Cl_2 \rightarrow RCl + SO_2Cl^{\bullet} \quad (1)$$

$$SO_2Cl^{\bullet} \leftrightharpoons SO_2 + Cl^{\bullet} \quad (2)$$

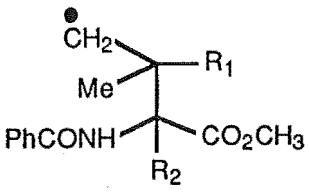
$$RH + SO_2Cl^{\bullet} \rightarrow R^{\bullet} + HCl + SO_2 \quad (3)$$

$$RH + Cl^{\bullet} \rightarrow R^{\bullet} + HCl \quad (4)$$

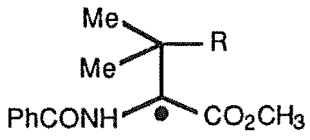
It is thought that an equilibrium exists between chlorine atom, sulphur dioxide and chlorosulphonyl radical (eq. 2), and that the latter radical is more selective in hydrogen atom transfer reactions than chlorine atom. Generally, the products of chlorinations with sulphuryl chloride may be attributed to hydrogen atom abstraction followed by halogen atom incorporation at the site of hydrogen atom abstraction. Thus production of the β-chlorovaline derivative (30a) and the diastereoisomers of the γ-chlorovaline derivative (31a) in the reaction of (29a) with sulphuryl chloride indicated that hydrogen atom abstraction from the β- and γ-positions of the valine derivative (29a) occurred to give the radicals (32a) and (33a). It would appear therefore, that the reaction of the valine derivative (29a) *via* regioselective β-carbon-hydrogen bond homolysis established the chemical validity of the hydrogen atom transfer reactions, (5) to give (12) and (19) to give (22), proposed in penicillin biosynthesis and in the β-hydroxylation of valine derivatives, respectively.



(32)
(a) R = H
(b) R = D



(33)
(a) $R_1 = R_2 = H$
(b) $R_1 = D$, $R_2 = H$
(c) $R_1 = H$, $R_2 = D$

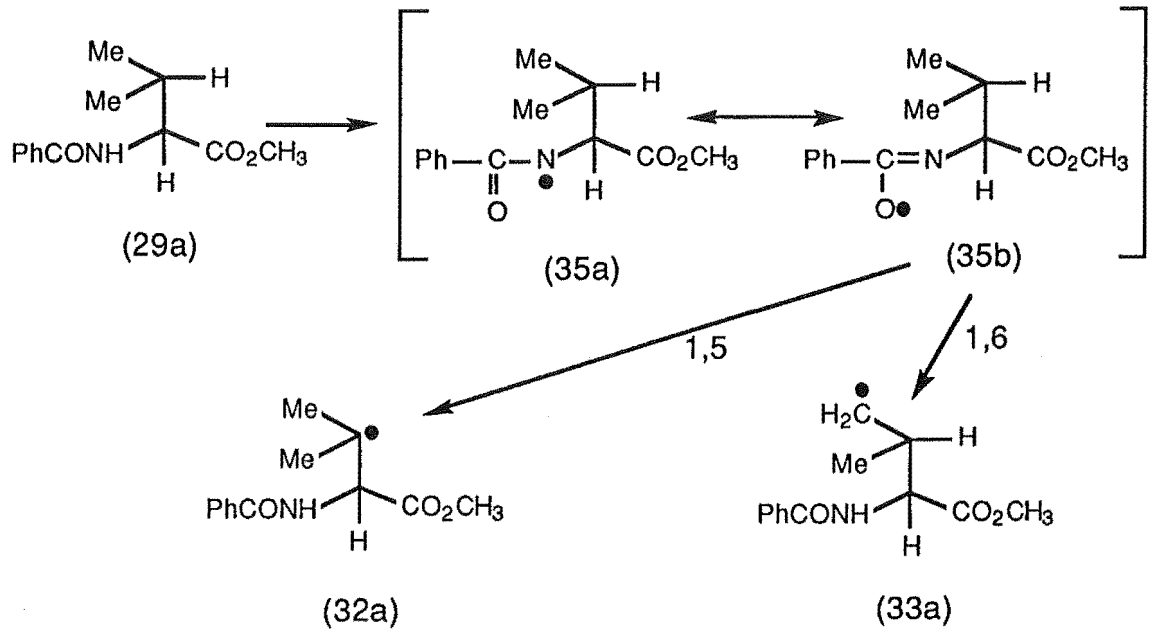


(34)
(a) R = H
(b) R = D

The contrast between the reactions of (23) and (26), *via* the corresponding α-centred radicals (24) and (27), and the reaction of (29a) with sulphuryl chloride remained anamolous. Investigation of this anomaly was one of the aims of this thesis.

Alternative explanations for the regioselective chlorination of the valine derivative (29a) to give the β-chlorovaline derivative (30a) and lesser amounts of

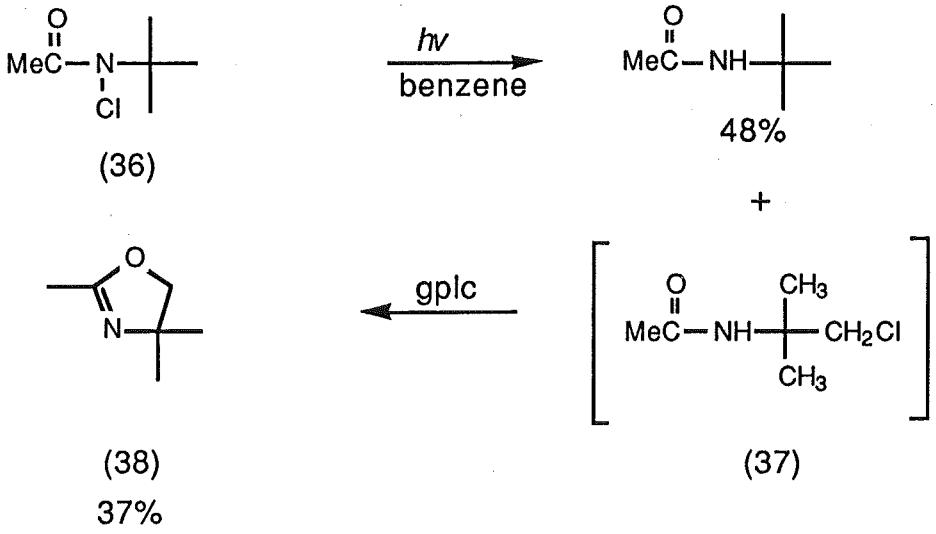
the γ-chlorovaline derivative (31a) can be considered. For example, the reaction could involve the amido radical (35a) as an intermediate (Scheme 8). Intramolecular hydrogen atom transfer to the nitrogen centred radical (35a) is unlikely to occur because of geometrical constraints.[39,40] Reaction, however, could possibly occur *via* the oxygen centred radical (35b) with intramolecular 1,5-hydrogen atom abstraction to produce the β-centred radical (32a). Hydrogen atom abstraction by the amide oxygen would involve the same size transition state, a six-membered ring, as that preferred by ordinary alkoxy radicals.[42] Reaction by a less facile intramolecular 1,6-hydrogen atom transfer to the amide oxygen would result in reaction at the γ-position to give the γ-centred radical (33a). An intramolecular hydrogen atom abstraction *via* the amido radical (35) in the chlorination of the valine derivative (29a) would invalidate the system as a model for the regioselective hydrogen atom transfers (5) to give (12) and (19) to give (22).



Scheme 8.

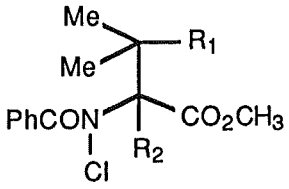
The possibility of intramolecular 1,5-hydrogen atom transfer to amide oxygen has been investigated.[41,42] Reaction of N-*t* -butyl-N-chloroacetamide (36) gave the oxazoline (38), derived from the β-chloroamide (37) (Scheme 9).

This was consistent with intramolecular 1,5-hydrogen atom transfer to the amide oxygen. Two independent groups of workers[43,44,45] have concluded, however, that this and related reactions did not involve intramolecular hydrogen atom abstraction. Evidence from e.s.r. spectroscopy indicated that the unpaired electron of the amido radical was not extensively delocalised to the carbonyl oxygen.[46]



Scheme 9.

Photolyses of the N-chlorovaline derivatives (39a-c) were examined to investigate reactions of the amido radical (35) and to probe for intramolecular hydrogen atom transfer to the oxygen-centred radical (35b). These results are



(39)
(a) $R_1 = R_2 = H$
(b) $R_1 = D, R_2 = H$
(c) $R_1 = H, R_2 = D$

presented in Chapter 1, together with a comparative study of the reaction of the valine derivative (29a), and it's deuterated analogues (29b) and (29c), with sulphuryl chloride.

It was anticipated that mechanistic information could be obtained through the measurement of deuterium isotope effects. For a primary deuterium isotope effect to be observed in a reaction involving a carbon-deuterium bond, the bond between the carbon and the deuterium has to be broken or formed during the rate determining step. Carbon-hydrogen and carbon-deuterium bonds have a zero point energy difference of around 4.8 kJ mol^{-1} because of the effect of mass on the stretching and bending frequencies of the bond. At room temperature nearly all the molecules are present in the zero point vibrational energy level.

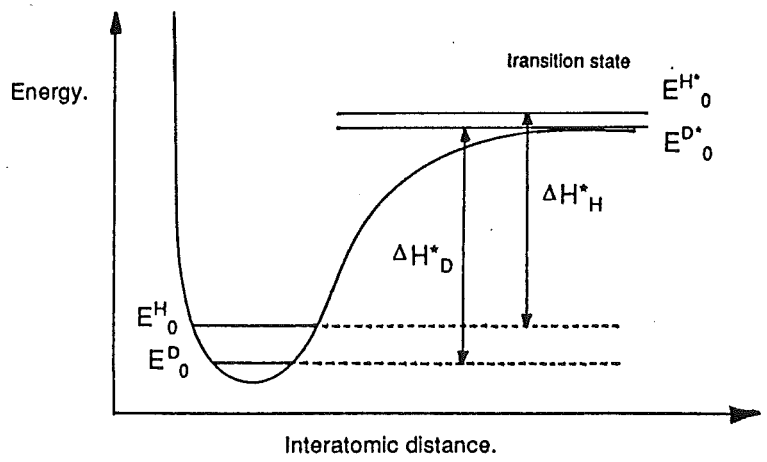


Figure 1.

The difference in the energy levels is depicted in Figure 1 where ΔH_H^* represents the energy required for formation of the [C ··· H ··· R] transition state and ΔH_D^* represents the energy required for formation of the [C ··· D ··· R] transition state. The carbon-hydrogen bond has a lower enthalpy of activation than the carbon-deuterium bond and dissociates at a greater rate. The Arrhenius equation, k = A.exp (-E/RT), may be substituted to give equation (5) which shows that the difference in zero point energy levels in the carbon-hydrogen and carbon-deuterium bonds is reflected in the rates of bond homolysis.

$$k_H / k_D = A_H / A_D . \exp [(E_0^H - E_0^D) / RT] \qquad (5)$$

For a reaction involving homolytic substitution...

$$C—H + R^{\bullet} \rightarrow [C \cdots H \cdots R] \qquad (6)$$

the zero point energies of the [C ··· H ··· R] and [C ··· D ··· R] transition states have to be considered. Thus, equation (5) becomes...

$$k_H / k_D = A_H / A_D . \exp [\Delta E_0 / RT] \qquad (7)$$

where...

$$\Delta E_0 = (E_0^H - E_0^D) — (E_0^{H^*} - E_0^{D^*}) \qquad (8)$$

The shape of the transition state has an effect on the magnitude of the deuterium isotope effect. In a symmetrical stretching of the transition state, the force constants for the two stretching modes become approximately the same and the central atom, hydrogen or deuterium, is relatively motionless. The symmetric stretching frequency becomes insensitive to the mass of the central atom. Hence there is no difference in the zero point energies of the [C ··· H ··· R] and [C ··· D ··· R] transition states, *ie.*, $(E_0^{H^*} - E_0^{D^*}) = 0$ and equation (7) reduces to equation (5). Thus, the difference in the zero point energies of the carbon-hydrogen and carbon-deuterium bonds in the reactants, *ie.*, $(E_0^H - E_0^D)$, gives rise to the maximum primary kinetic deuterium isotope effect, which is around 7. If the transition state is not symmetrical, eg., if it possesses minimal bond breaking or extensive bond breaking, or involves a bent transition state, the stretching vibration is only partly lost. Hence the difference in the zero point energies of the [C ··· H ··· R] and [C ··· D ··· R] transition states, *ie* $(E_0^{H^*} - E_0^{D^*})$, reduces the magnitude of ΔE_0 and a lowered primary kinetic deuterium isotope effect results. Extremely reactive radicals, the reactions of which have a relatively low activation energy, react *via* a transition state with minimal bond breaking and hence a low deuterium isotope effect is observed. As the reactivity of the radical or substrate is decreased, the deuterium isotope effect increases with the activation energy until a more symmetrical transition state is reached. Rigourous quantitative derivations of this general description have been examined previously.[47,48,49]

For the work described in Chapter 1 and thereafter in the thesis, it was necessary to measure the relative rates of atom transfer reactions of compounds. These measurements can be made by reacting mixtures of two or more substrates with a common reactant and determining the relative rates of consumption of each substrate in the mixture. In such an experiment reactions of the following type are involved.[50]

$$R_1X + Z^\bullet \xrightarrow{k_1} R_1^\bullet + XZ \qquad (9)$$

$$R_2Y + Z^\bullet \xrightarrow{k_2} R_2^\bullet + YZ \qquad (10)$$

Provided neither R_1X nor R_2Y is produced in the reaction, either by the reverse process of equations (9) and (10) or by other processes, it follows that...

$$\delta(R_1X) / \delta t = k_1.[R_1X].[Z^\bullet] \qquad (11)$$

$$\delta(R_2Y) / \delta t = k_2.[R_2Y].[Z^\bullet]. \qquad (12)$$

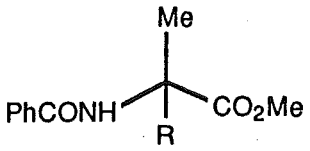
Thus...

$$\delta(R_1X) / \delta(R_2Y) = k_1 / k_2.[R_1X] / [R_2Y]. \qquad (13)$$

Integration gives...

$$k_1/k_2 = \ln ([R_1X]_t / [R_1X]_0) / \ln ([R_2Y]_t / [R_2Y]_0) \qquad (14)$$

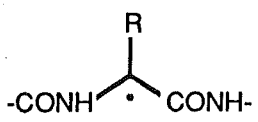
To investigate the general nature of atom transfer reactions of valine derivatives, the study of the photolyses of (39a-c) and of the reactions of (29a-c) with sulphuryl chloride was extended to include an investigation of reactions of the valine derivatives (29a-c) with N-bromosuccinimide (NBS) and di-*t*-butyl peroxide. This work was augmented with studies of reactions of the alanine derivative (40) with sulphuryl chloride and NBS. The results are discussed in Chapter 2 of the thesis.



(40)
(a) R = H
(b) R = D

Proteins are thought to be one of the crucial or key sites of biological damage resulting from the absorption of photochemical radiation and from reaction with adventitious radicals produced in cellular processes.[51] Transient free radical species have been identified in a wide variety of proteins upon irradiation.[52] The radicals identified may be categorised into three types which reflect the amino acid composition of the protein. These are aromatic radicals, sulphur radicals and aliphatic radicals.

The presence of aromatic residues, such as tryptophan and phenyl-alanine, leads to the formation of aromatic radicals.[53] These are thought to be involved as sensitisers for adjacent sulphur containing residues.[54] Sulphur radicals have been identified, and their formation is very efficient. This is possibly because of energy transfer from photosensitisers such as aromatic residues. The formation of sulphur radicals in proteins is likely to be important because the integrity of the disulphide linkage is crucial in the maintenance of the tertiary protein structure.[55] The third type of radical produced from the photolysis of proteins is the α-carbon-centred radical (41).



(41)
(a) R = H

Irradiation experiments with polycrystalline and single crystal samples of amino acids and dipeptides have produced two main types of radicals as shown

by e.s.r. spectroscopy.[56] One of these gives e.s.r. spectra which are broad and anisotropic, called the "sulphur pattern", mainly because similar spectra were observed for a number of thiols and disulphides. The e.s.r. spectra of the other type of radical show a doublet resonance, characteristic of resonances found for dipeptides such as glycylglycine and N-acetylglycine. Early work on the dipeptides showed that the radical responsible for the doublet resonance was formed by a specific hydrogen abstraction of an α-hydrogen.[57] It was suggested that an α-carbon-centred radical (41a) was formed in proteins with the unpaired electron centred mainly in a π-orbital on an α-carbon. Indeed, molecular orbital calculations of the distribution of the unpaired spin density in N-acetylglycyl radical have shown it to be distributed over the molecule in p-orbitals perpendicular to the plane of the molecule as shown in Fig. 2.[55] It can be noted that both α-spin (positive values) and β-spin (negative values) contribute. The main contribution to the electron density is from the α-centre with some contribution from the carboxyl group and the amide groups. That the unpaired electron density is calculated to be delocalised to the amide and carboxy substituents is in accord with the description of the amidocarboxy-substituted captodative radicals given above.

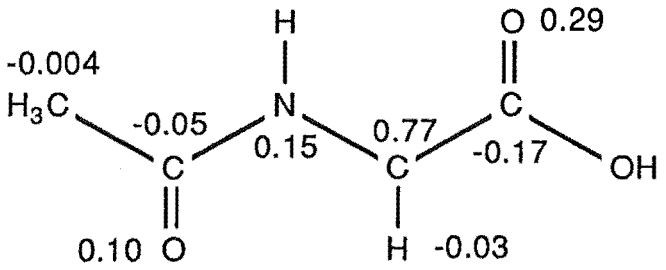
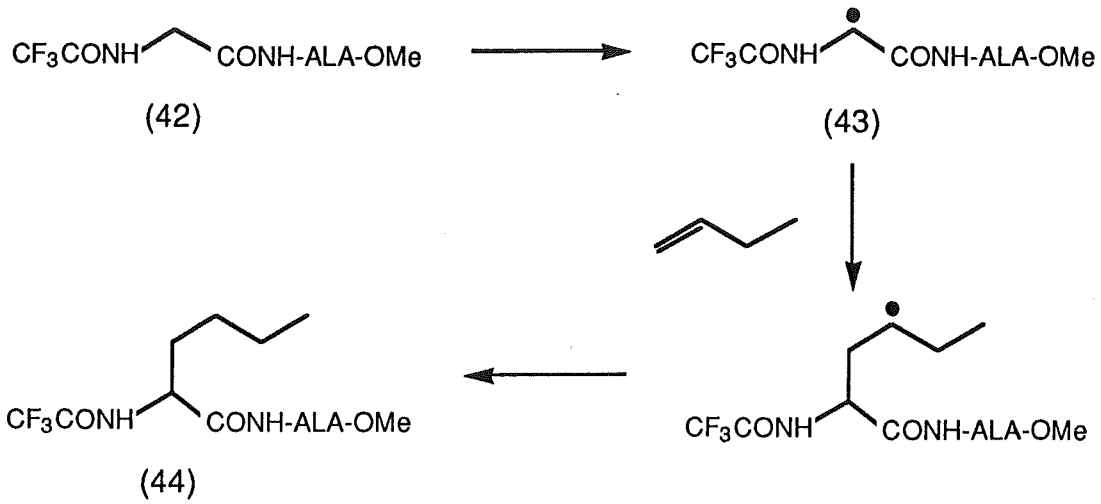


Figure 2.

Reports of the photoalkylation of peptides[58,59] and proteins[59] through the preferential reactivity of glycine residues have emphasised the importance of formation of the α-centred radical (41a). In these experiments initiation was

usually achieved through photoexcitation of a ketone, which then abstracted a peptide α-hydrogen atom. The radical was subsequently "scavenged" by an olefin to yield the branched α-amino acid derivative. As an example, formation of the α-centred radical (43) from the glycine residue of the dipeptide (42) by reaction with triplet acetone was followed by addition to 1-butene, and hydrogen atom incorporation to give the norleucine residue (44) (Scheme 10).

Reports of this preferential reactivity of glycine residues in free radical reactions of proteins, peptides and other amino acid derivatives have been attributed to selective hydrogen atom abstraction from the α-carbon of the glycine residues.[58,59] This selectivity is contrary to the expectation that tertiary radicals should be formed in preference to secondary radicals.[60] Glycine residues afford secondary radicals by α-carbon-hydrogen bond homolysis, whereas analogous reactions of derivatives of other amino acids produce tertiary radicals.



Scheme 10.

To investigate this seemingly anamolous reactivity of the glycine residues it was decided to extend the study of the atom transfer reactions of valine derivatives to include the analogous reactions of other amino acid derivatives. This work is presented in Chapter 3 of the thesis.

In Chapter 4 of the thesis preliminary experiments in the synthesis of

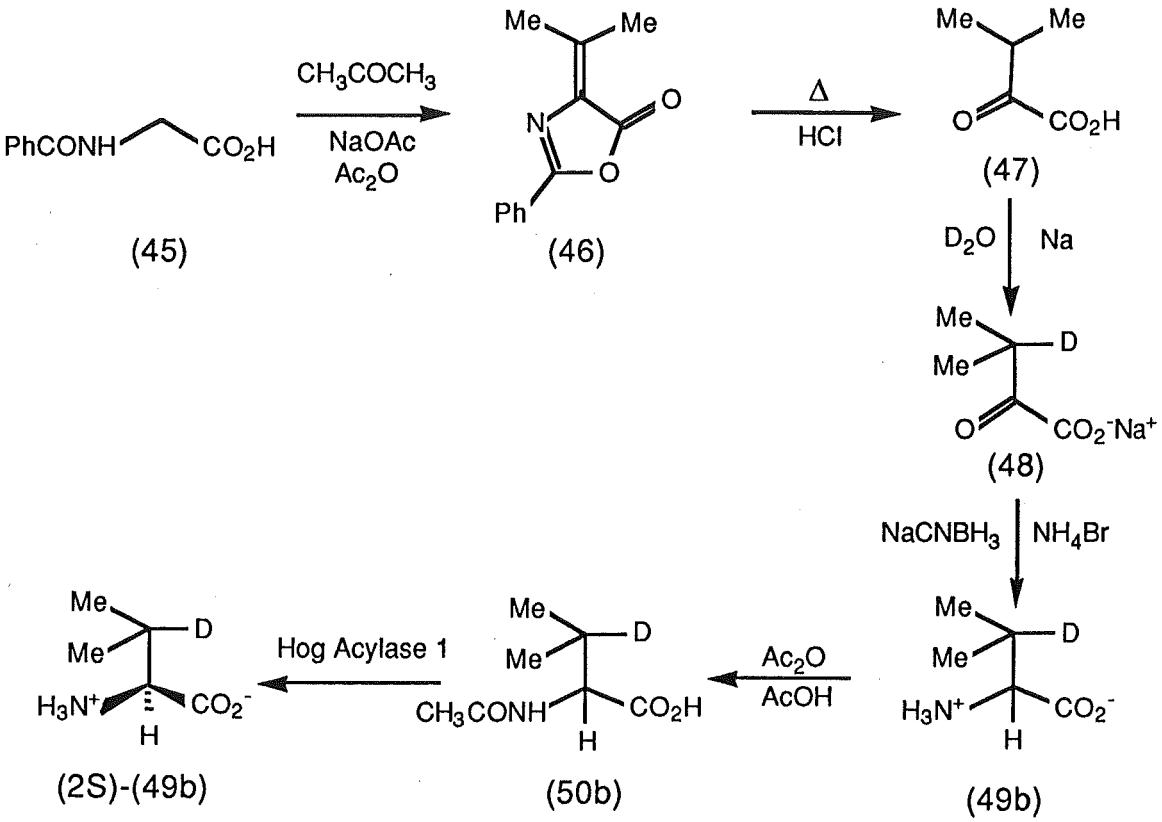
modified amino acid derivatives are described. These experiments are designed to exploit the results of the work described in Chapters 1-3.

CHAPTER 1.

REGIOSELECTIVE CHLORINATION OF VALINE DERIVATIVES.

To investigate the possible involvement of the amido radical (35a) as an intermediate in the regioselective chlorination of the valine derivative (29a) to give (30a) and (31a) (Scheme 8), and to investigate the possibility of intramolecular 1,5-hydrogen atom transfer to the amide oxygen, reactions of the N-chlorovaline derivatives (39a-c) were studied. The N-chlorovaline derivatives (39a-c) were obtained as shown in Schemes 11-14.

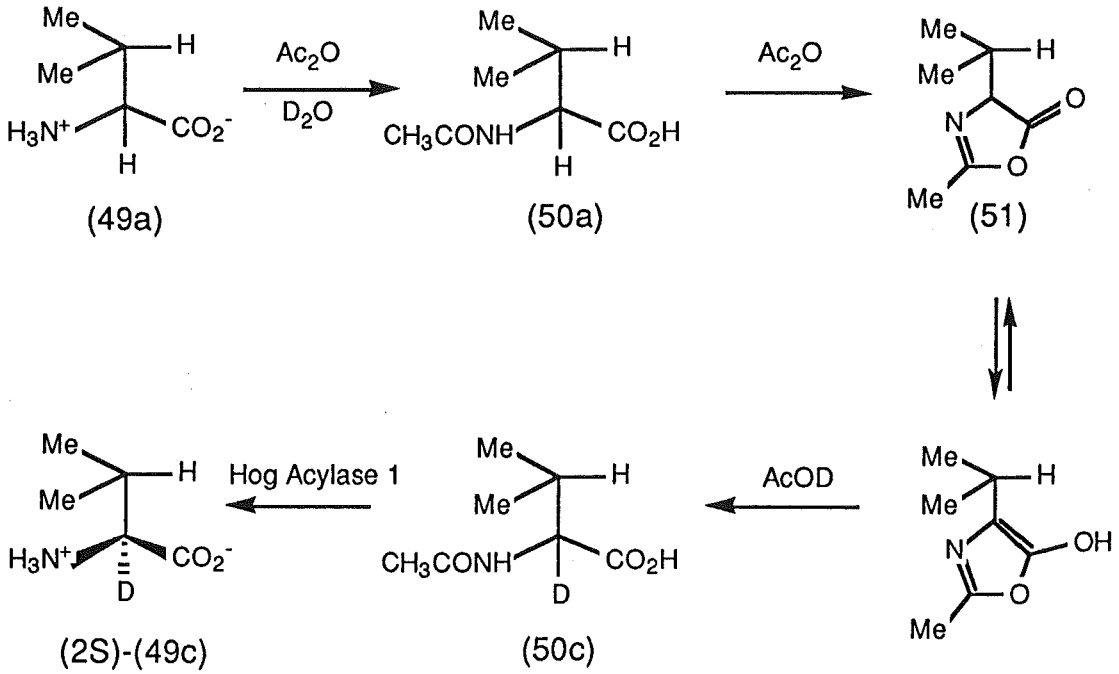
(2S)-[3-^{2}H]-Valine (49b) was prepared as shown in Scheme 11. Dimethylpyruvic acid (47) was prepared according to the method of Ramage and Simonson.[61] Hippuric acid (45) and acetone, heated with acetic anhydride and sodium acetate gave the crude 2-phenyl-4-isopropylidene oxazolone (46) which was treated with conc. hydrochloric acid to give dimethylpyruvic acid (47).



Scheme 11.

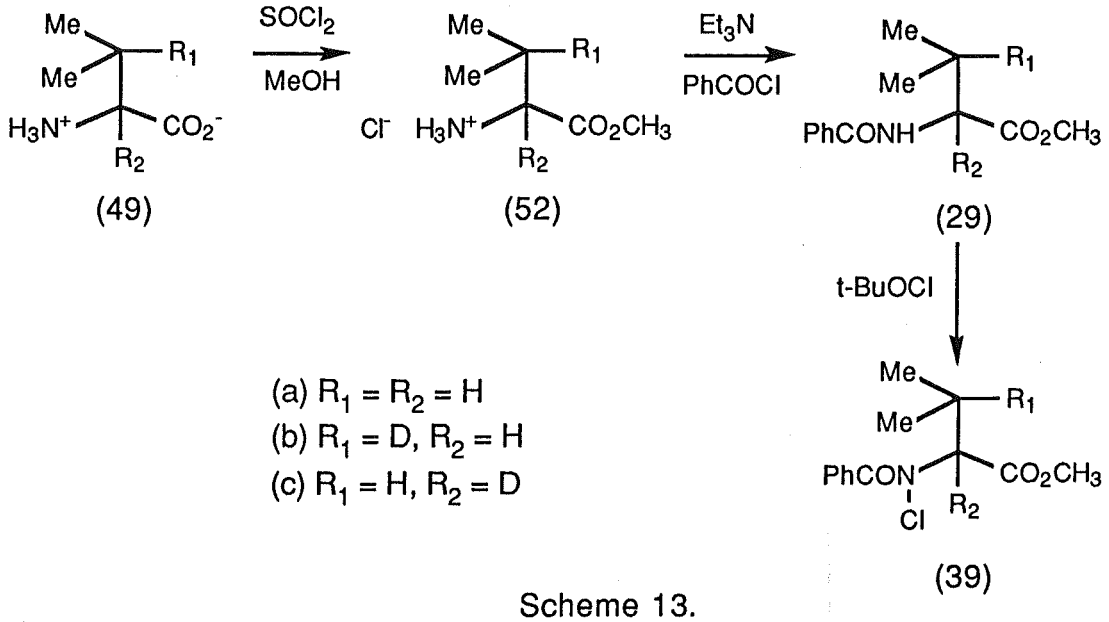
(2S)-[3-^{2}H]-Valine (49b) was prepared according to the method of Baldwin and Wan.[62] Dimethylpyruvic acid (47) was converted to the sodium salt and treated with sodium deuteroxide in D_2O. The crude sodium α-keto-β-deuteroisovalerate (48) was reductively aminated with ammonium bromide and sodium cyanoborohydride in methanol and the product, [3-^{2}H]-valine (49b), crystallised from ethanol / aniline. [3-^{2}H]-Valine (49b) was resolved by selective enzymic deacylation of N-acetyl-(2S)-[3-^{2}H]-valine (50b). [3-^{2}H]-Valine (49b) was heated at reflux with acetic anhydride in glacial acetic acid to give the crude N-acetyl-[3-^{2}H]-valine (50b). A neutral aqueous solution of N-acetyl-[3-^{2}H]-valine (50b) was incubated with Hog Acylase I overnight. The suspension was boiled briefly with a pinch of activated charcoal to deactivate the enzyme which was removed by filtration. The recovered (2S)-[3-^{2}H]-valine (49b) crystallised from water / ethanol.

(2S)-[2-^{2}H]-Valine (49c) was prepared according to the methods of Baldwin[62] and Greenstein[63] (Scheme 12). Deuterium was incorporated at the



Scheme 12.

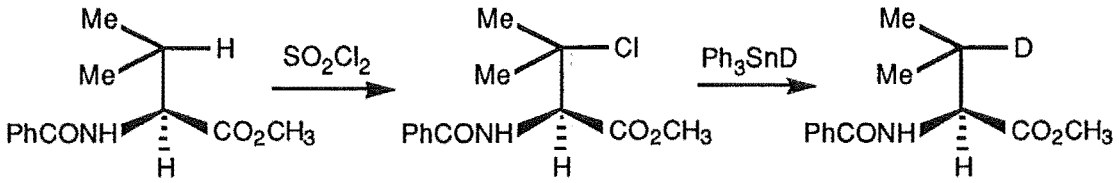
α-position as a result of the racemisation of the azlactone (51), formed by treatment of valine (49a) with more than two equivalents of acetic anhydride in acetic acid-OD. Hence, valine (49a) was heated at reflux with acetic anhydride to which D_2O had been added. More D_2O was added to convert the remaining acetic anhydride to acetic acid-OD, the solution cooled and the solvent removed. A neutral solution of the product N-acetyl-[2-^{2}H]-valine (50c) was incubated overnight with Hog Acylase I, the enzyme was then deactivated and removed. The recovered (2S)-[2-^{2}H]-valine (49c) crystallised from water / ethanol. The valines (49a-c) were derivatised using standard procedures described by Applewhite *et. al.* [64] (Scheme 13). Typically, valine (49a) was dissolved in methanol to which thionyl chloride had been added and the solution was left to stand overnight. The solvent was removed *in vacuo* to give the crude valine methyl ester hydrochloride (52) which was suspended in ethyl acetate, then treated with triethylamine and benzoyl chloride. Subsequent work up and chromatography afforded the valine derivative (29a). This method was used to prepare the valine derivatives (2R,S)-(29a), (2R)-(29a), (2S)-(29b), and (2S)-(29c). Alternatively the (2S)-valine derivative (29b) was prepared directly from



(a) $R_1 = R_2 = H$
(b) $R_1 = D$, $R_2 = H$
(c) $R_1 = H$, $R_2 = D$

Scheme 13.

the (2S)-valine derivative (29a) (Scheme 14). Reaction of (2S)-(29a) with sulphuryl chloride gave the β-chlorovaline derivative (30a). Treatment of the β-chlorovaline derivative (30a) with triphenyltin deuteride in benzene gave the (2S)-valine derivative (29b).[65] Triphenyltin deuteride was prepared by the reduction of triphenyltin chloride with lithium aluminium deuteride in dry ether.[66] The enantiomers (2R)-(29a), (2S)-(29b), and (2S)-(29c) were analysed by g.l.c. using a Chrompack XE-60-S-VAL-S-X-PEA capillary column. Comparison of the enantiomers of (29a-c) with a sample of (2R,S)-(29a) showed that the chiral purity of each of the enantiomers was greater than 99%.



(2S)-(29a) (30a) (2S)-(29b)

Scheme 14.

The N-chlorovaline derivatives (39a-c) were prepared by treatment of the corresponding valine derivatives (29a-c) with *t*-butylhypochlorite, prepared by bubbling chlorine through a basic solution of *t*-butanol[67] (Scheme 13). A solution of each N-chlorovaline derivative (39a), (39b) or (39c) in benzene was photolysed in a Rayonet Photochemical Reactor with 3000Å light at approximately 25°C. Photolyses of the N-chlorovaline derivatives (39) afforded the β-chlorovaline derivative (30) and diastereoisomers of the γ-chlorovaline derivative (31) as well as considerable amounts of the valine derivative (29). The product mixtures were analysed by h.p.l.c. and the product ratios determined by integration of the h.p.l.c. traces. H.p.l.c. analysis using a UV detector involved the use of the benzamido group as a chromophore. It was assumed that the products (30a-b) and (31a-c) had sufficiently similar response factors for the UV

detector to allow direct determination of the relative amounts of the compounds present.

Whereas the reaction of the N-chlorovaline derivative (39a) afforded the β-chlorovaline derivative (30a) and the γ-chlorovaline derivative (31a) in the ratio *ca.* 2.9:1.0, reaction of the β-deuterated analogue (39b) under identical conditions gave (30a) and (31b) in the ratio *ca.* 1.6:1.0. Reaction of the α-deuterated analogue (39c) gave (30b) and (31c) in the ratio *ca.* 2.9:1.0 (Table 1).

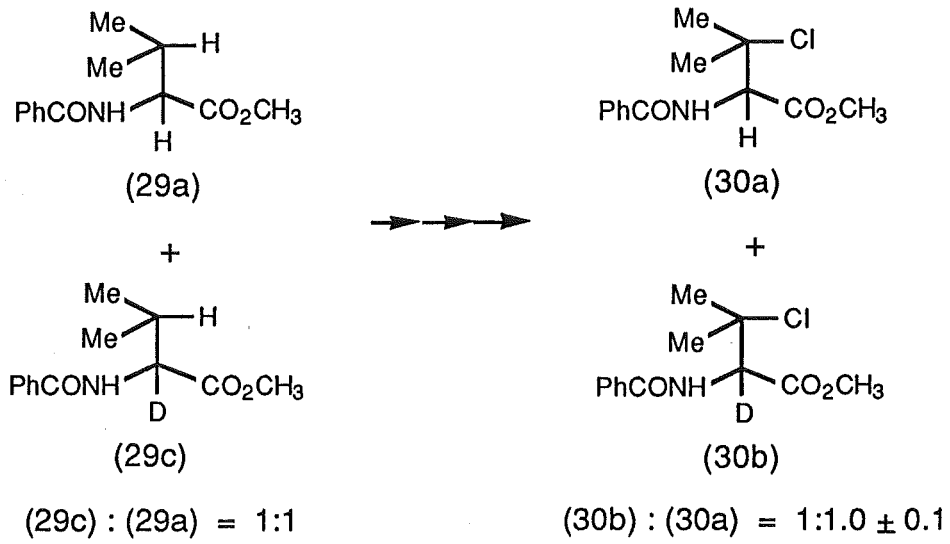
The reactions of (39a-c) were carried out under identical conditions, therefore it may be assumed that the rate of formation of the γ-chlorovaline derivative (31) is approximately the same in each reaction. The presence of a deuterium atom at the α- or β-position of the N-chlorovaline derivatives (39b) or (39c) may give rise to a secondary deuterium isotope effect for abstraction of a γ-hydrogen atom. The magnitude of such an effect is usually very small,[47,68,69] $k_H / k_D \approx 1.1$, therefore any such effect is reasonably ignored in the determination of a primary deuterium isotope effect. The ratios of the products formed in the photolyses of (39a-c) therefore show a deuterium isotope effect of 1.8 ± 0.18 for β-carbon-hydrogen bond homolysis but no deuterium isotope for α-carbon-hydrogen bond homolysis.

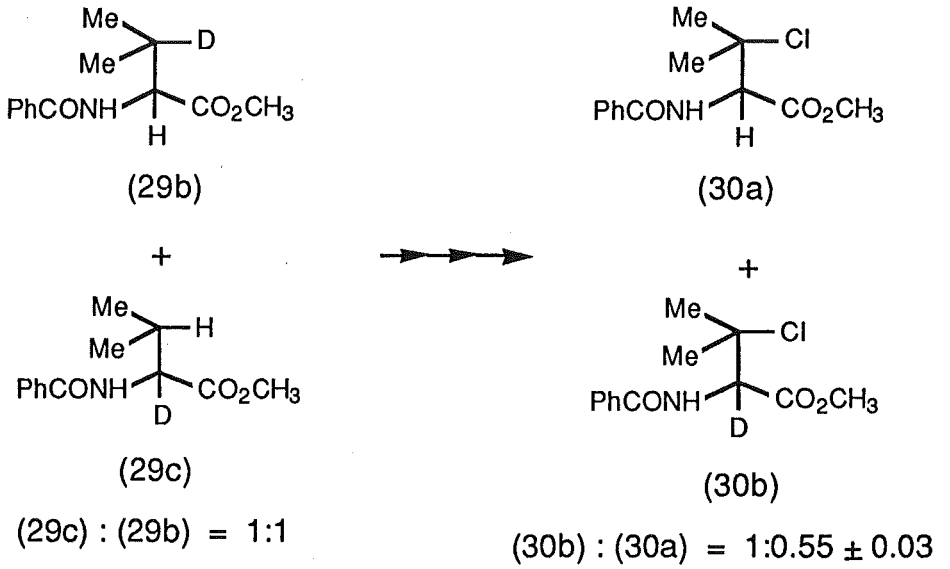
Table 1. Products of the photolyses of the N-chlorovaline derivatives (39a-c).

Reactant	(39a)	(39b)	(39c)
Products	(30a):(31a)	(30a):(31b)	(30c):(31c)
Product Ratios	$2.9 \pm 0.1 : 1.0$	$1.6 \pm 0.1 : 1.0$	$2.9 \pm 0.1 : 1.0$

An alternative measurement of the deuterium isotope effect in the reaction of (39b) was made using ^1H n.m.r. spectroscopy. The relative yields of the β-

chlorovaline derivative (30a) produced from the reactions of the N-chlorovaline derivative (39a) and the β-deuterated analogue (39b) were measured using the reaction of the α-deuterated analogue (39c) to give (30b) as a reference.





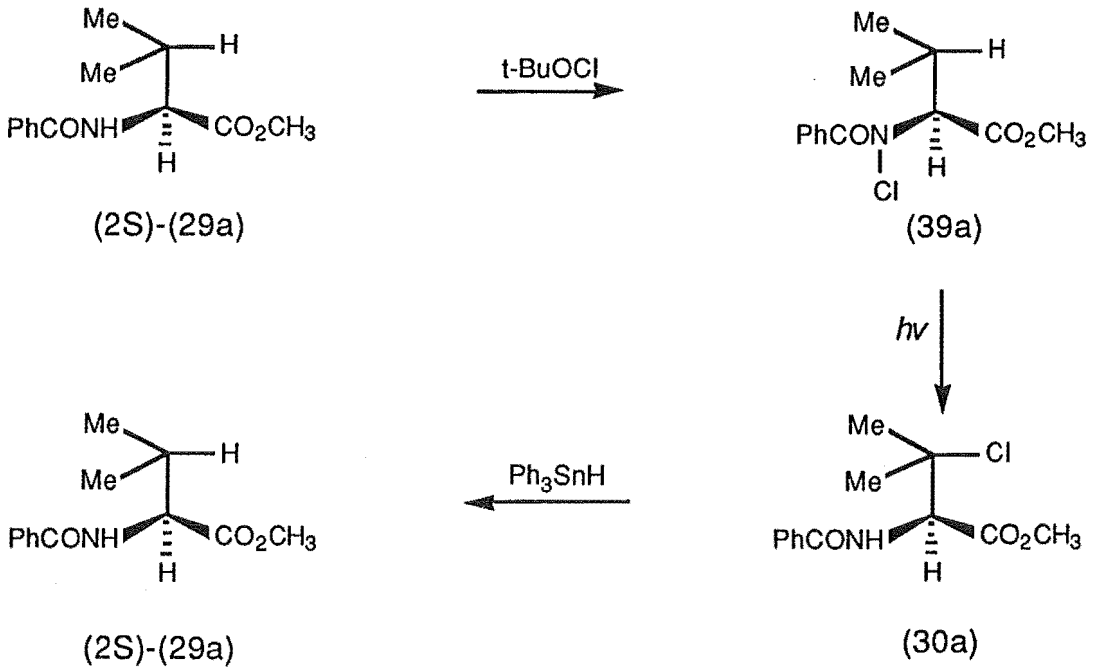
Scheme 15.

A mixture of the N-chlorovaline derivatives (39a) and (39c), obtained by treating a 1:1 mixture of (29a) and (29c) with *t*-butylhypochlorite, was irradiated under identical conditions to the above photolyses. Purification by column chromatography and preparative h.p.l.c. yielded a mixture of the β-chlorovaline

derivatives (30a) and (30b). The starting mixture of (29a) and (29c) and the product mixture of (30a) and (30b) were examined by ^1H n.m.r. This experiment was repeated for a 1:1 mixture of (39b) and (39c) produced from (29b) and (29c). The rate of reaction of (39c) to give (30b) was assumed to be the same in each experiment, hence this was used to compare the rates of reaction of (39a) and (39b). Again the effect of any secondary deuterium isotope effects on the rate of reactions of (39a-c) was assumed to be negligible.

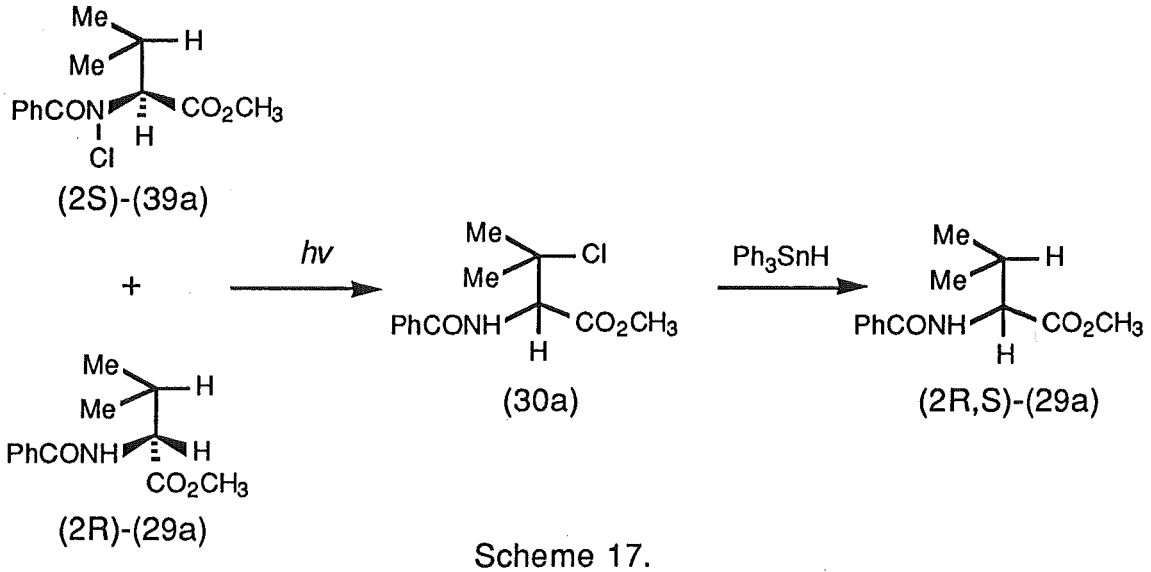
^1H N.m.r. spectroscopy was used to monitor the ratio of the integrals of the methyl ester protons to α-protons in starting material and product. Integration of the ^1H n.m.r. spectrum of the 1:1 mixture of the valine derivatives (29a) and (29c) gave a ratio of 6:1 for methyl ester protons to α-protons. Integration of the ^1H n.m.r. spectrum of the mixture of β-chlorovaline derivatives (30a) and (30b), isolated by h.p.l.c. from the photolysis mixture, gave a ratio of 6:1 for methyl ester protons to α-protons. Integration of the ^1H n.m.r. spectrum of the 1:1 mixture of the valine derivatives (29b) and (29c) gave a ratio of 6:1 for methyl ester protons to α-protons. Integration of the ^1H n.m.r. spectrum of the mixture of β-chloro-valine derivatives (30a) and (30b), isolated by h.p.l.c. from the photolysis mixture, gave a ratio of 11:1 for methyl ester protons to α-protons. The difference between the ratios of methyl ester protons to α-protons in the product mixtures is a measurement of the relative rates of production of the β-chlorovaline derivative (30a), from the N-chlorovaline derivatives (39a) and (39b). This difference in the rates of production of the β-chlorovaline derivative (30a) reflects the different rates of abstraction of the β-hydrogen and β-deuterium in the N-chlorovaline derivatives (39a) and (39b). The deuterium isotope effect of 1.83 determined by this method for the reaction of (39a) and (39b) is, within experimental error, the same as the value obtained by the alternative method described above.
The deuterium isotope effect shows that homolysis of the β-carbon-hydrogen bond is the rate determining step in the reaction of the N-chlorovaline derivative (39a) to give (30a). However, either an intramolecular or an intermolecular

mechanism could give a deuterium isotope effect for β-carbon-hydrogen bond homolysis. To determine whether the reaction of the N-chlorovaline derivative (39a) involves an intramolecular or intermolecular hydrogen atom transfer the chirality of the compounds was utilised. When the (2S)-valine derivative (29a) was used to prepare the N-chlorovaline derivative (39a), photolysis gave the β-chlorovaline derivative (30a) which was isolated by column chromatography and preparative h.p.l.c. (Scheme 16). Treatment of the purified β-chlorovaline



Scheme 16.

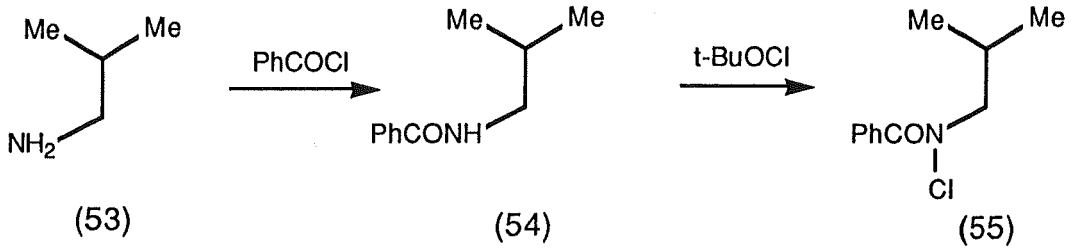
derivative (30a) with triphenyltin hydride in benzene afforded (2S)-(29a). Triphenyltin hydride was prepared by the reduction of triphenyltin chloride with an excess of lithium aluminium hydride.[70] The product valine derivative (29a) was analysed by g.l.c. and found to be pure (2S)-enantiomer (29a). A mixture of the N-chlorovaline derivative (39a) synthesised from the (2S)-valine derivative (29a), and the (2R)-valine derivative (29a) in the ratio of 1.07:1.0, was photolysed in benzene, as described above (Scheme 17). Separation of the products by



Scheme 17.

chromatography gave the β-chlorovaline derivative (30a) which was treated with triphenyltin hydride. The product, the valine derivative (29a), was analysed by g.l.c. and found to be a mixture of (2S)-(29a) and (2R)-(29a) in the ratio of 1.28:1.0.

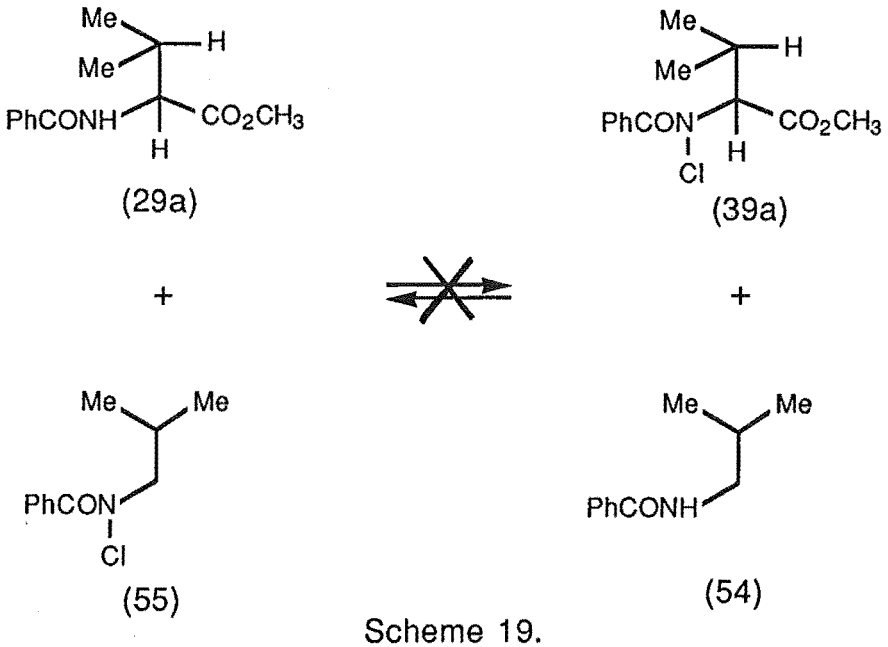
N-*iso*-Butylbenzamide (54) was prepared by the reaction of *iso*-butylamine (53) and benzoyl chloride, in the presence of triethylamine (Scheme 18). Treatment of N-*iso*-butylbenzamide (54) with *t*-butylhypochlorite as described above gave N-*iso*-butyl-N-chlorobenzamide (55). A mixture of the valine



Scheme 18.

derivative (29a) and N-*iso*-butyl-N-chlorobenzamide (55) was photolysed as described above and the reaction course followed by ^{1}H n.m.r. spectroscopy (Scheme 19). The initial ^{1}H n.m.r. spectrum indicated a distinct quartet for the α-

hydrogen of the valine derivative (29a). Subsequent spectra showed no evidence of formation of a doublet indicative of the α-hydrogen in the N-chlorovaline derivative (39a). A mixture of the N-chlorovaline derivative (39a) and N-*iso*-butylbenzamide (54) was photolysed under the same reaction conditions and the reaction course followed by ^1H n.m.r. There was no evidence for formation of N-*iso*-butyl-N-chlorobenzamide (55). Thus, no interconversion of the N-chloroamide (55) and the valine derivative (29a) to give the N-chlorovaline derivative (39a) and N-*iso*-butylbenzamide (54) or *vice versa* was observed under the reaction conditions.

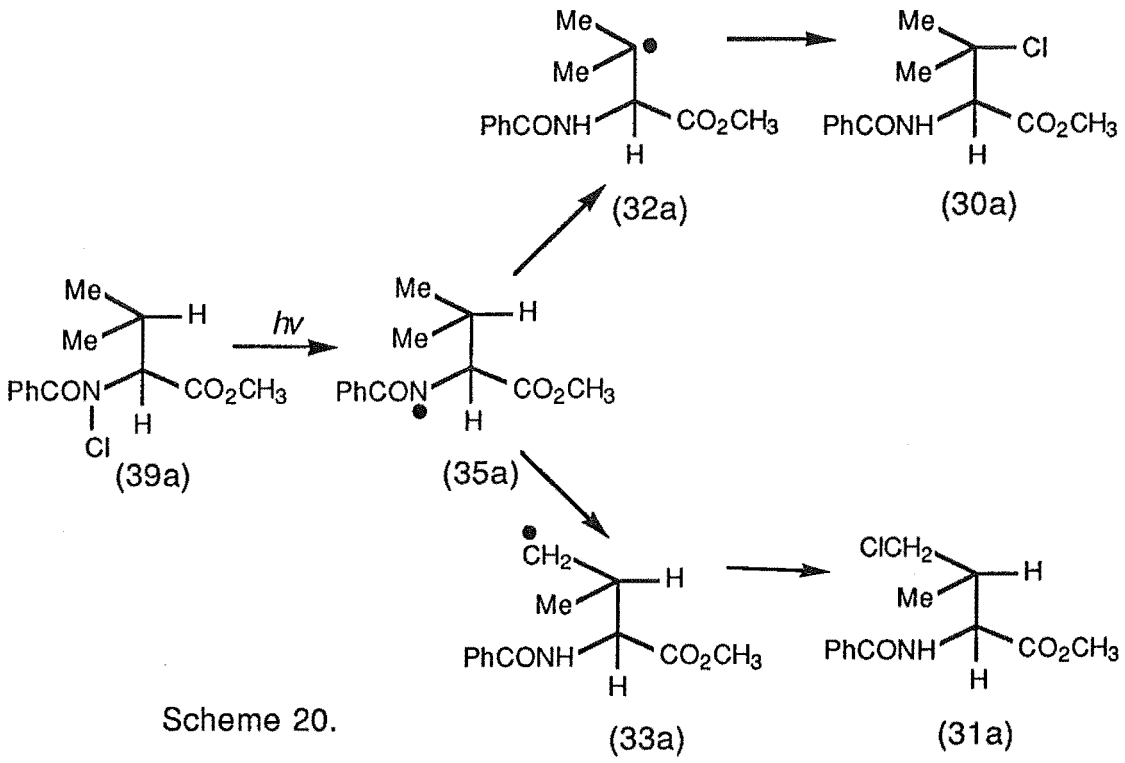


Scheme 19.

Production of the N-chlorovaline derivative (39a) from the (2S)-valine derivative (29a), formation of the β-chlorovaline derivative (30a), and it s subsequent reduction with triphenyltin hydride to give (29a), proceeded without racemisation at the α-position (Scheme 16). Hence the racemic product formed in the photolysis of (39a) in the presence of the (2R)-valine derivative (29a) (Scheme 17) must result through interaction with (29a). Since there was no interconversion of the N-chloroamide (55) and the valine derivative (29a)

to give the N-chlorovaline derivative (39a) and N-*iso* -butylbenzamide (54) or *vice versa* (Scheme 19) under the reaction conditions, the possibility of racemisation by a chlorine atom transfer between the (2R)-valine derivative (29a) and the N-chlorovaline derivative (39a) is excluded. An intramolecular hydrogen atom transfer in a reaction of the enantiomeric starting material (39a) would be expected to produce an enantiomeric product. The observed racemisation indicates an intermolecular hydrogen atom transfer, presumably to the amidyl radical (35a) from both the (2S)-N-chlorovaline derivative (39a) and the (2R)-valine derivative (29a).

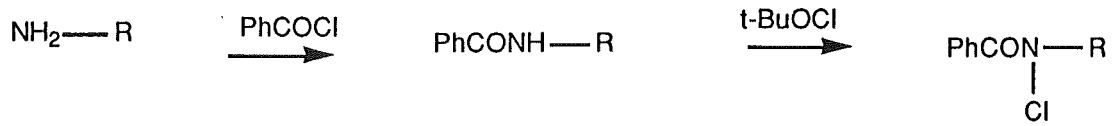
Thus, reaction of the N-chlorovaline derivative (39a) appears to proceed *via* formation of the amidyl radical (35a) and involve intermolecular hydrogen atom abstraction to give the radicals (32a) and (33a), precursors of the chlorinated valine derivatives (30a) and (31a), respectively (Scheme 20).



Scheme 20.

Although the possibility of some intramolecular hydrogen atom transfer to the oxygen-centred radical (35b) can not be excluded, it is clear that the intermolecular hydrogen atom transfer to the nitrogen-centred radical (35a) is the predominant process, even in the dilute solutions used in the experiments.

Other examples of intermolecular chlorination in the photolyses of N-chloroamides were observed in the reactions of the valine derivative (29a) with the N-chloroamides (58a) and (58b). N-*t*-butylbenzamide (57a), prepared by the reaction of *t*-butylamine (56a) and benzoyl chloride, was treated with *t*-butylhypochlorite as described above to give N-*t*-butyl-N-chlorobenzamide (58a) (Scheme 21). N-Phenylbenzamide (57b), prepared similarly from freshly distilled aniline and benzoyl chloride, was treated with *t*-butylhypochlorite as described above to give N-chloro-N-phenylbenzamide (58b) (Scheme 21). Photolysis of mixtures of the valine derivative (29a) and N-*t*-butyl-N-chlorobenzamide (58a) or N-chloro-N-phenylbenzamide (58b) in benzene afforded the β-chlorovaline derivative (30a) and the γ-chlorovaline derivative (31a).



(56) (57) (58)

(a) R = t-Bu
(b) R = Ph

Scheme 21.

In light of the evidence that the amidyl radical (35a) reacts by intermolecular hydrogen abstraction, the regioselective formation of the β-chlorovaline derivative (30a) and the γ-chlorovaline derivative (31a) in the reaction of the valine derivative (29a) with sulphuryl chloride and in the photolyses of the N-chloroamide can not be attributed to intramolecular reactions of the amidyl radical (35a) as an intermediate. The unexplained regioselectivity observed in the reaction of the valine derivative with sulphuryl chloride was

investigated further. The chirality of the valine derivatives (2R)-(29a), (2S)-(29b) and (2S)-(29c) was exploited in these studies.

Treatment of N-benzoylvaline methyl ester (29a) with sulphuryl chloride in carbon tetrachloride or benzene at reflux under nitrogen, as described,[36] afforded mixtures of the β-chlorovaline derivative (30a) and diastereoisomers of the γ-chlorovaline derivative (31a). The combined yield of the products (30a) and (31a) was high provided the extent of reaction of (29a) was less than 80%. More extensive reaction resulted in the decomposition of the primary products. H.p.l.c. analysis of crude reaction mixtures at 10-20% reaction showed the parent amide (29a), the β-chlorovaline derivative (30a), the γ-chlorovaline derivative (31a) and no other products.

Treatment of the β-chlorovaline derivative (30a), produced from the reaction of (29a) with sulphuryl chloride, with triphenyltin hydride gave the valine derivative (29a). When the (2S)-valine derivative (29a) was treated as described, analysis by g.l.c. showed the product to be the pure enantiomer (2S)-(29a).

Deuterium isotope effects were determined for reactions of the valine derivatives (29a-c) with sulphuryl chloride. This was achieved by measuring the relative rates of production of the β-chlorovaline derivative (30) and the γ-chlorovaline derivative (31). In carbon tetrachloride reaction of the valine derivative (29a) with sulphuryl chloride gave the β-chlorovaline derivative (30a) and the γ-chlorovaline derivative (31a) in the ratio of *ca.* 1:1. This represents a selectivity, on a per hydrogen basis, of 6:1 for tertiary carbon-hydrogen bond homolysis over primary carbon-hydrogen bond homolysis. In benzene the same reaction gave the β-chlorovaline derivative (30a) and the γ-chlorovaline derivative (31a) in the ratio of *ca.* 1.75:1. This represents a selectivity, on a per hydrogen basis, of 10.5:1 for tertiary carbon-hydrogen bond homolysis over primary carbon-hydrogen bond homolysis. Solutions of each of the valine derivatives (29a), (29b) or (29c) in benzene were treated with sulphuryl chloride

and a trace of benzoyl peroxide. The mixtures were heated at reflux under nitrogen. The product mixtures were analysed by h.p.l.c. and the ratios of the β-chlorovaline derivative (30) to the γ-chlorovaline derivative (31) determined by integration of the h.p.l.c. traces (Table 2). Whereas the reaction of the valine derivative (29a) afforded the β-chlorovaline derivative (30a) and the γ-chlorovaline derivative (31a) in the ratio *ca.* 1.75:1.0, reaction of (29b) under identical conditions gave (30a) and (23b) in the ratio *ca.* 1.10:1.0. Reaction of (29c) gave (30b) and (23c) in the ratio *ca.* 1.75:1.0. The rate of formation of the γ-chlorovaline derivatives (31a-c) from the respective valine derivatives (29a-c) may be assumed to be approximately the same in each case because the reactions were carried out under identical conditions and any secondary deuterium isotope effect is likely to be negligible.

Thus, the relative rates of production of (30a-b) in the reaction of (29a-c) with sulphuryl chloride show a deuterium isotope effect for β-carbon-hydrogen bond homolysis but no deuterium isotope effect for α-carbon-hydrogen bond homolysis.

Table 2. Products of reaction of the valine derivatives (29a-c) with sulphuryl chloride in benzene.

Reactant	(29a)	(29b)	(29c)
Products	(30a):(31a)	(30a):(31b)	(30b):(31c)
Product Ratio	1.75 ± 0.05: 1.0	1.10 ± 0.05: 1.0	1.75 ± 0.05: 1.0

Another assessment of the deuterium isotope effect observed above was made by measuring the relative rates of reaction of the valine derivatives (29a-c) with sulphuryl chloride in benzene in competitive experiments. It was possible to measure the relative rates of reaction of (29a-c) using a mixture of the (2R)-valine derivative (29a) and the (2S)-valine derivative (29b), and a mixture of

(2R)-(29a) and (2S)-(29c). The chiralty of the valine derivatives (29a-c) was exploited in the analysis of the mixtures. Enantiomers exhibit identical reactivity in reactions with sulphuryl chloride but are physically separable for analysis by g.l.c. using a Chrompak XE-60-S-VAL-S-X-PEA column. *t*-Butylbenzamide was used as an internal standard to measure the extent of reaction. The starting and product mixtures were analysed by g.l.c. and the initial and final ratios of the valine derivatives (29a-c) measured.

The relative rates of reaction of (29a-c) were calculated using equation (14) (Table 3). Abstraction of hydrogen or deuterium from (29a-c) must be irreversible for equation (14) to be valid. ^{1}H N.m.r. analyses of the valine derivative (29b) at 40-50% reaction showed no loss of deuterium from the starting material, hence reaction at the β-position appears to be irreversible. Reaction of (2S)-valine derivative (29a) with sulphuryl chloride occurred without racemisation at the α-position as determined by g.l.c. analysis. A reversible reaction at the α-position would be expected to result in racemisation at that position.

Whereas the β-deuterated derivative (29b) reacted with sulphuryl chloride *ca.* 0.80 times as fast as the unlabelled valine derivative (29a), the α-deuterated valine derivative (29c) and the unlabelled valine derivative (29a) reacted at the same rate. Thus, the relative rates of reaction of (29a-c) with sulphuryl chloride in benzene show a deuterium isotope effect for β-carbon-hydrogen bond homolysis but no deuterium isotope effect for α-carbon-hydrogen bond homolysis.

Table 3. Relative rates of reaction of (29a-c) with sulphuryl chloride in benzene.

Starting material	(29a)	(29b)	(29c)
k_{rel}	1.0*	0.80 ± 0.04	1.0 ± 0.03

*Assigned as unity.

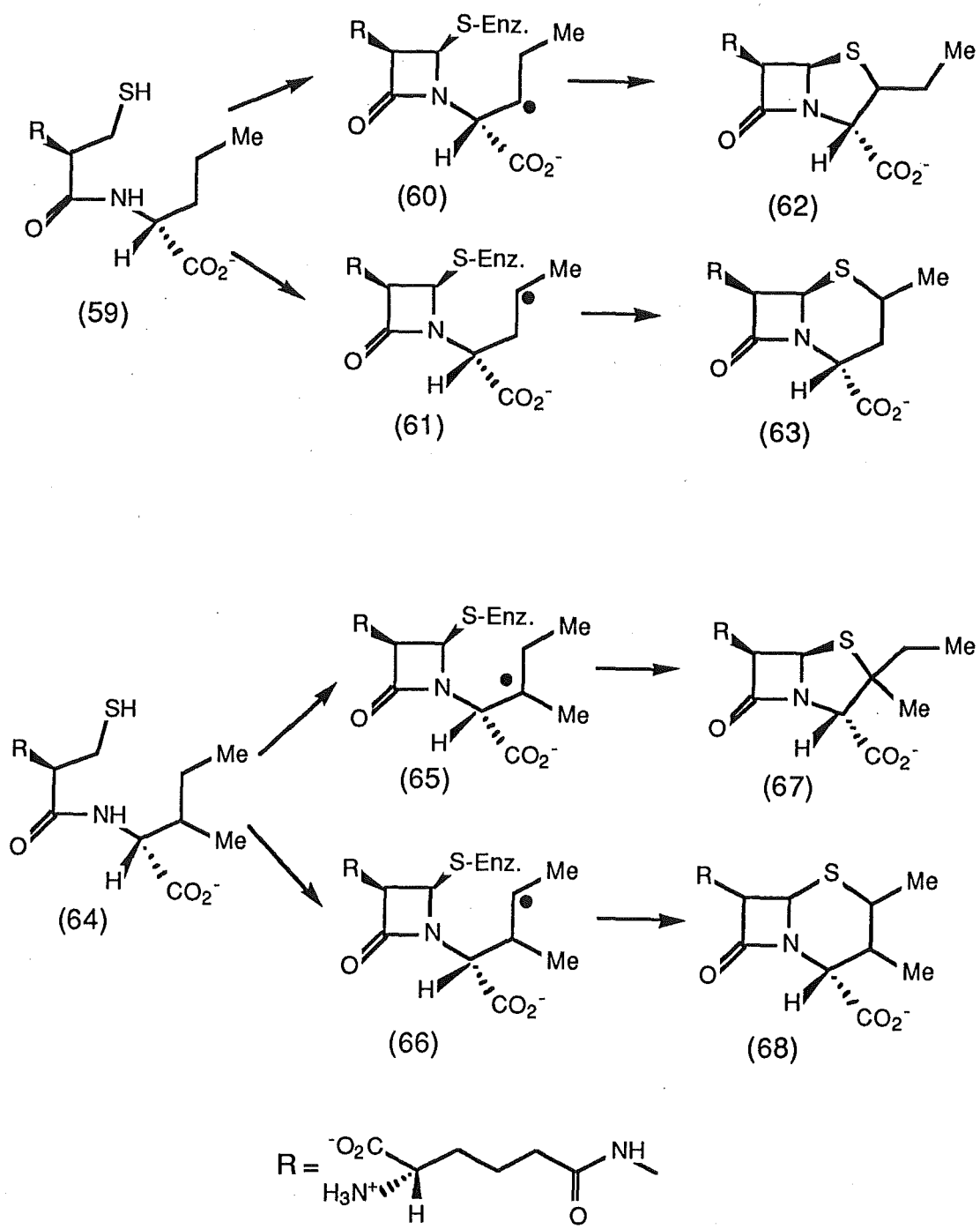
Two methods have shown a deuterium isotope effect for β-carbon-hydrogen bond homolysis, hence the reaction of (29a) with sulphuryl chloride in benzene may be attributed to intermolecular hydrogen atom transfer. This indicates reaction of (29a) proceeds by hydrogen atom abstraction to give (32a) followed by chlorine atom incorporation to give (30a). If it is assumed that (31a) is formed by intermolecular hydrogen atom abstraction from the γ-position of (29a) to give (33a) with subsequent chlorine atom incorporation, then the deuterium isotope effects measured by the two methods are within experimental error. On the basis of the product ratios (30a) + (31a) / (30a) + (31b) from (29a) and (29b) and assuming the rate of production of (31a) and (31b) is the same in each case, then by equation (15) $k_{(29a)} / k_{(29b)} = 1.3 \pm 0.1$. The value is, within experimental error, the same as the value obtained for the product study of *ca.* 1.25 ± 0.06.

$$k_{(29a)} / k_{(29b)} = (30a) + (31a) / (30a) + (31b)$$
$$= (1.75 + 1.0) / (1.10 + 1.0) = 1.31 \pm 0.1 \qquad (15)$$

Further evidence for intermolecular hydrogen atom abstraction in the reactions of (29a) with sulphuryl chloride comes from the solvent effect observed in these reactions. The selectivity of 6:1 for β-carbon-hydrogen bond homolysis *versus* γ-carbon-hydrogen bond homolysis for reaction in carbon tetrachloride increased to 10.5:1 for reaction in benzene. Similar solvent effects have been observed before and have been explained in terms of π-complexation of the abstracting radical in benzene solvent.[71,72] The solvent effect may be attributed to complexation of the chlorine atom to benzene, leading to enhanced selectivity in the intermolecular hydrogen atom abstraction from (29a). It is difficult to rationalise the solvent effect in terms of an intramolecular hydrogen atom transfer.

Thus the evidence presented above indicates that the reaction of (29a) with sulphuryl chloride proceeds *via* intermolecular hydrogen atom abstraction to give the radicals (32a) and (33a) which incorporate chlorine atom to give (30a) and (31a) respectively. That the reaction of the valine derivative (29a) with sulphuryl chloride does not involve reaction at the α-position has been shown. There was no deuterium isotope effect for α-carbon-hydrogen bond homolysis and no evidence for products resulting from reaction at the α-position. Formation of the β-chlorovaline derivative (30a) occurred without racemisation at the α-position. The tertiary radical (32a) and the primary radical (33a) are formed in preference to the captodative radical (34a).

Regioselective hydrogen atom abstraction in the reaction of the valine derivative (29a) with sulphuryl chloride to give the radical (32a) is consistent with the proposed regioselective hydrogen atom abstractions (5) to give (1b) in penicillin biosynthesis, and (19) to give (22) in the β-hydroxylation of valine residues. During the course of this work Baldwin *et. al.* [11,12] have reported studies of the interaction of isopenicillin N synthetase enzyme with modified substrates (Scheme 22). Reactions of modified substrates such as δ-(*S*-α-aminoadipoyl-*S*-cysteinyl-*R*-norvaline) (59) and δ-(*S*-α-aminoadipoyl-*S*-cysteinyl-*R*-(-)-isoleucine) (64) with isopenicillin N synthetase demonstrate a balance between penam and cepham biosynthesis which may be attributed to steric and radical stability effects.[12] In the reaction of δ-(*S*-α-aminoadipoyl-*S*-cysteinyl-*R*-norvaline) (59) there is a possibility of formation of two secondary radicals, at C-3 (60) and C-4 (61) of the C-terminal residue. In this case formation of the cepham ring (63) predominates over formation of the penam ring (62), presumably due to steric effects. In the case of δ-(*S*-α-aminoadipoyl-*S*-cysteinyl-*R*-(-)-isoleucine) (64), formation of the penam ring (67) *via* the tertiary radical (65) competes with formation of the cepham ring (68) *via* the secondary radical (66).



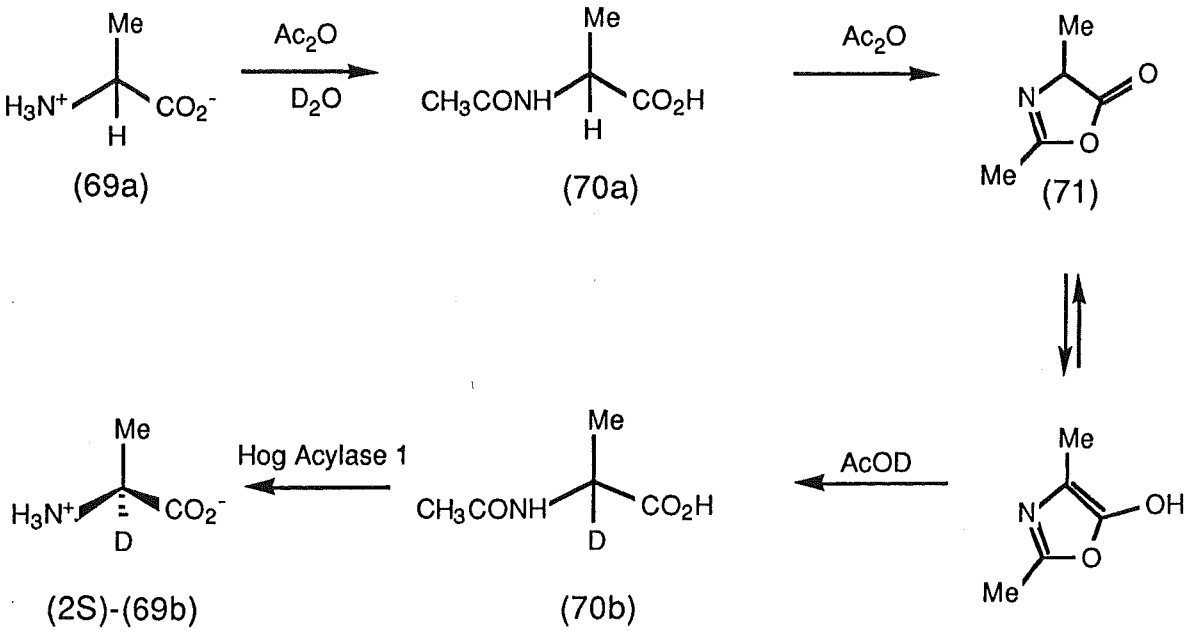
Scheme 22.

These studies, and the work presented above, support the previous contention[13] that abstraction of the β-valinyl hydrogen atom in penicillin biosynthesis is a homolytic process. The work presented in this chapter shows that abstraction of the β-valinyl hydrogen from species analogous to (5) and (19) may occur despite the expected relative stabilities of β-centred radicals such as (12) and (22) compared to the corresponding α-centred radicals such as (20) and (21).

CHAPTER 2.

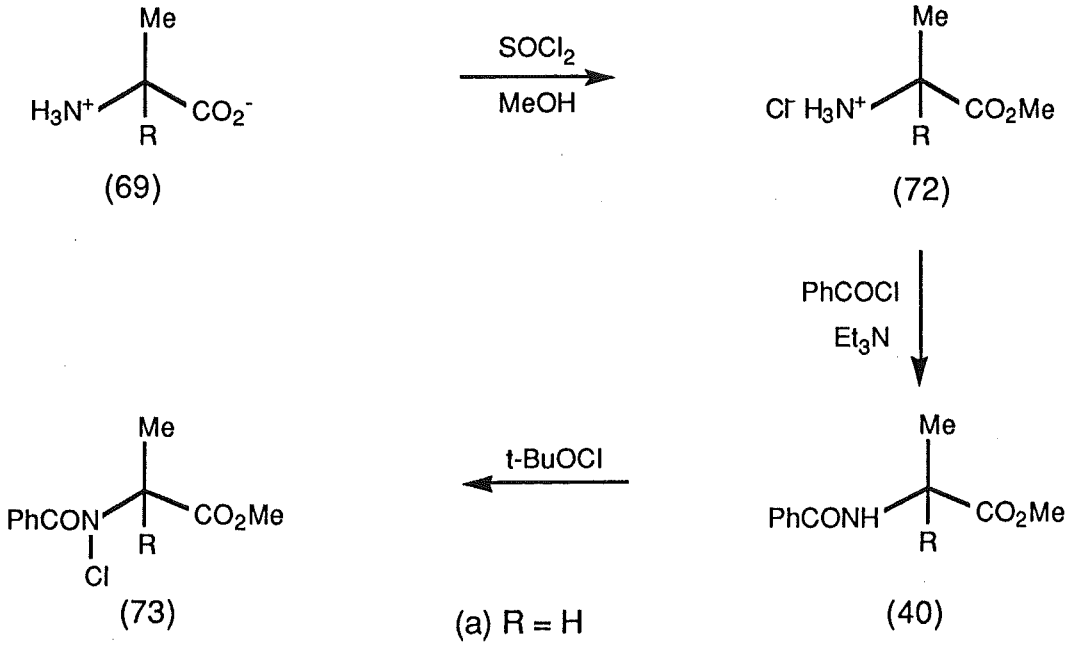
REGIOSELECTIVE FORMATION OF AMIDOCARBOXY-SUBSTITUTED FREE RADICALS.

While the results described in Chapter 1 indicate that the reaction of (29a) with sulphuryl chloride and the photolysis of (39a) involve regioselective intermolecular transfer of the respective β-valinyl hydrogens, they do not provide an explanation for the unexpected regioselectivity observed in these reactions. To gain further insight into these reactions and to investigate the general nature of atom transfer reactions of valine derivatives, reactions of N-benzoylvaline methyl ester (29a), and it's deuterated analogues (29b) and (29c), with N-bromosuccinimide and di-*t*-butyl peroxide were studied, in conjunction with reactions of N-benzoylalanine methyl ester (40) with sulphuryl chloride and NBS, and the reaction of the dichlorovaline derivative (75) with tri-*n*-butyltin hydride.
The syntheses of the valine derivatives (29a-c) have been discussed in Chapter 1. The alanine derivatives (40a) and (40b) were obtained as shown in Schemes 23 and 24.



Scheme 23.

(2S)-[2-^{2}H]-Alanine (69b) was prepared in a similar manner to (2S)-[2-^{2}H]-valine (49c). Deuterium was incorporated at the α-position as a result of the isomerisation of the azlactone (71), formed by treatment of alanine (69a) with more than two equivalents of acetic anhydride in acetic acid-OD. Selective enzymic deacylation of N-acetyl-(2S)-[2-^{2}H]-alanine (70b) afforded (2S)-[2-^{2}H]-alanine (69b). Hence, alanine (69a) was heated at reflux in acetic anhydride to which D_2O had been added. More D_2O was added to convert any remaining acetic anhydride to acetic acid-OD and the solvent removed *in vacuo* to give the crude N-acetyl-[2-^{2}H]-alanine (70b). A neutral aqueous solution of the crude N-acetyl-[2-^{2}H]-alanine (70b) was incubated with Hog Acylase I overnight at 37°. The enzyme was deactivated by brief boiling with activated charcoal and removed. The recovered (2S)-[2-^{2}H]-alanine (69b) crystallised from water / ethanol. The (2S)-[2-^{2}H]-alanine derivative (40b) and the (2R)-alanine derivative (40a) were prepared from (2S)-[2-^{2}H]-alanine (69b) and (2R)-alanine (69a) respectively, using standard methods[64] (Scheme 24). Typically, alanine (69a)

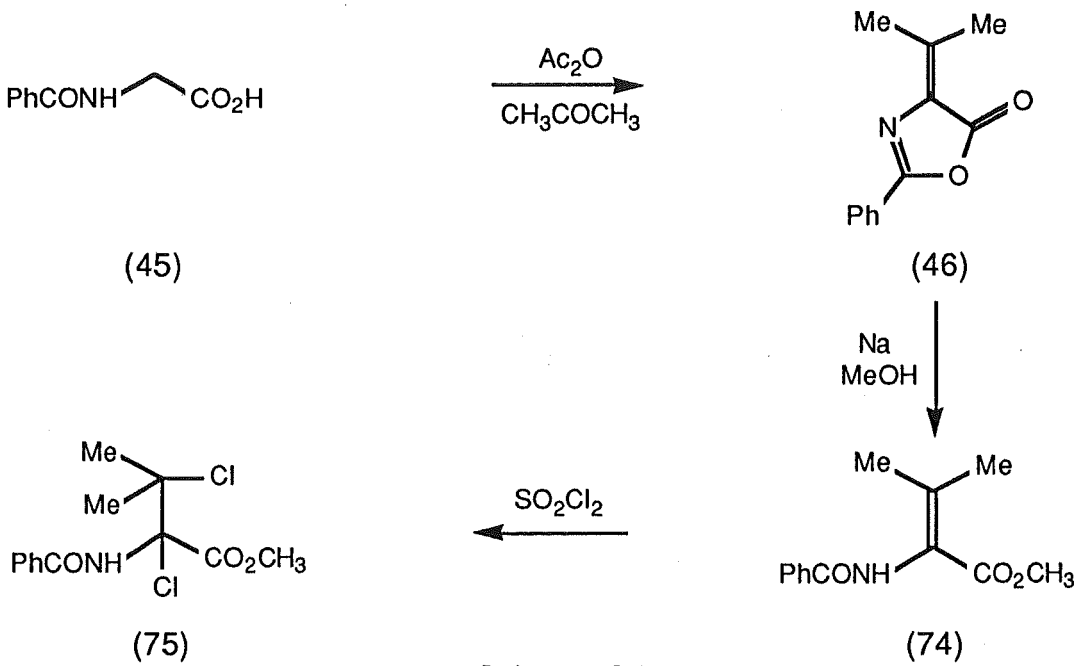


(a) R = H
(b) R = D

Scheme 24.

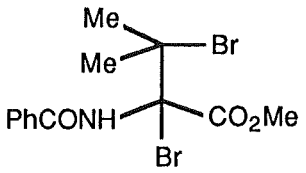
was dissolved in methanol to which thionyl chloride had been added and the solution stood overnight at room temperature. After removal of the solvent, the crude alanine methyl ester hydrochloride (72a) was dissolved in ethyl acetate and treated with triethylamine and benzoyl chloride. Work up followed by chromatography gave the alanine derivative (40a). The N-chloroalanine derivative (73) was prepared by the reaction of the alanine derivative (40a) with *t*-butylhypochlorite as described above for the N-chlorovaline derivatives (39a-c).

The acylenamine (74) was prepared according to the method of Ramage and Simonson[61] (Scheme 25). Treatment of hippuric acid (45) with acetone and acetic anhydride gave 2-phenyl-4-isopropylidene oxazolone (46) which was treated with sodium methoxide in methanol to yield the acylenamine (74). The dichlorovaline derivative (75) was obtained by reaction of the acylenamine (74) with sulphuryl chloride in carbon tetrachloride at room temperature.

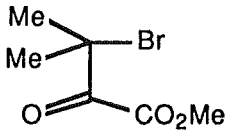


Scheme 25.

Reaction of the valine derivative (29a) with NBS was carried out in refluxing carbon tetrachloride under nitrogen, with photochemical initiation. Reaction of (29a) with three equivalents of NBS afforded, after chromatography, the dibromovaline derivative (76) in high yield. The dibromovaline derivative (76) was identified on the basis of it's ^{1}H n.m.r. spectrum and mass spectrum. Parent ions at *m/z* 395, 393 and 391 in a 1:2:1 ratio in the mass spectrum indicated a dibromovaline derivative. The ^{1}H n.m.r. spectrum indicated the presence of the two γ-methyl groups, the methyl ester protons and the benzamide group. The infrared spectrum showed the amido and ester groups were intact. Hydrogenolysis of the dibromovaline derivative (76) over palladium on carbon gave a mixture of the valine derivative (29a) and the acylenamine (74), which were separated by chromatography. The acylenamine (74) was identified by comparison with an authentic sample prepared according to the method of Ramage and Simonson.[61] Reaction of the acylenamine (74) with two equivalents of NBS in carbon tetrachloride afforded the dibromovaline derivative (76).



(76)



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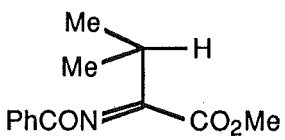
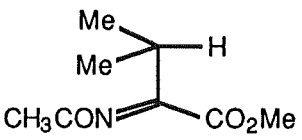


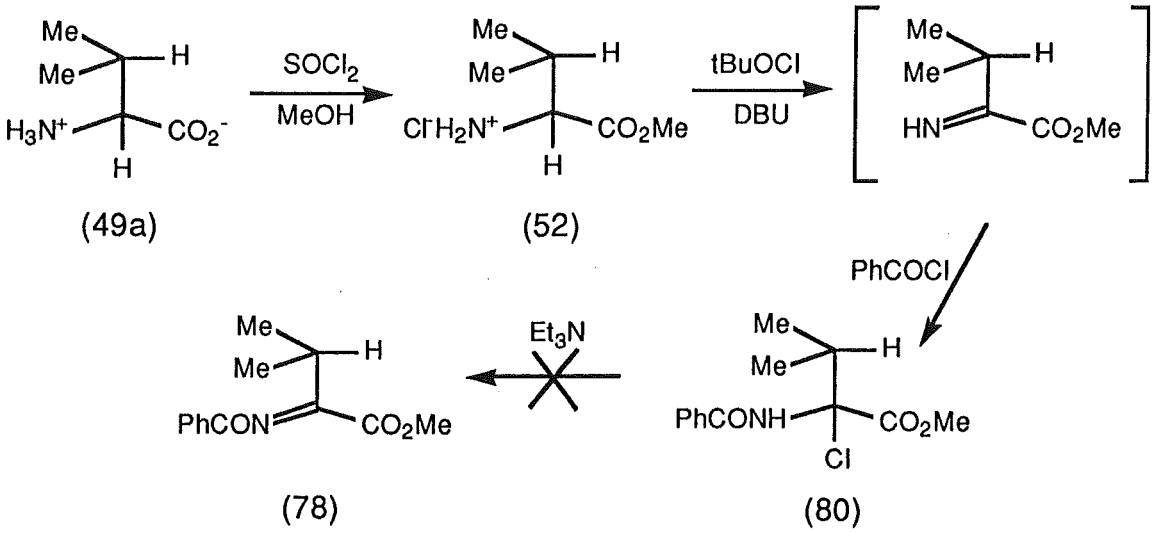
(78)



(79)

Reaction of the valine derivative (29a) with two equivalents of NBS afforded the β-bromo-α-ketoester (77). The structure of (77) was assigned on the basis of it's ^{1}H n.m.r. spectrum and mass spectrum. The ^{1}H n.m.r. spectrum

consisted of a singlet at δ 1.98 ppm for the γ-methyl protons and a singlet at δ 3.90 ppm for the methyl ester protons. The presence of parent ions in the mass spectrum at *m/z* 210 and 208 in a 1:1 ratio suggested a monobrominated compound. ^{1}H N.m.r. spectra of reaction mixtures where less than 20% of the valine derivative (29a) had reacted, showed a doublet resonance at δ 1.30 ppm (J = 7 Hz) which could not be resolved further. This is consistent with formation of the acylimine (78) as a reaction intermediate, where the doublet may be assigned to the γ-protons. The ^{1}H n.m.r. spectrum of the acylimine (79), prepared by Poisel,[73] showed a doublet at δ 1.16 ppm (J = 7 Hz) for the γ-protons. All attempts to isolate the intermediate (78) failed, and an attempt to synthesise an authentic sample of the acylimine (78) for comparison was not successful (Scheme 26).[73,74] This was attempted by treating valine (49a) with methanol to which thionyl chloride had been added, to produce valine methyl ester hydrochloride (52). Treatment of (52) with *t*-butylhypochlorite and 1,8-diazabicyclo[5.4.0]undec-7-ene, followed by benzoyl chloride produced the α-chlorovaline derivative (80). The α-chlorovaline derivative (80) was treated with triethylamine, but failed to give the acylimine (78) by dehydrochlorination.

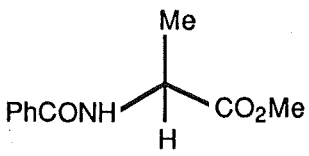


(49a) (52) (80) (78)

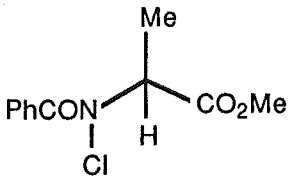
Scheme 26.

Trace amounts of the acylenamine (74) were detected in the reaction of the valine derivative (29a) with NBS, by ^1H n.m.r. spectroscopy and h.p.l.c. analysis.

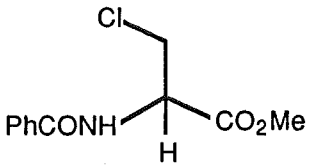
Treatment of N-benzoylalanine methyl ester (40a) with sulphuryl chloride in refluxing benzene under nitrogen, with a trace of benzoyl peroxide, afforded moderate amounts of the β-chloroalanine derivative (81), identified by comparison with an authentic sample obtained as described previously.[75] Photolysis of the N-chloroalanine derivative (73) in benzene in a Rayonet Photochemical Reactor gave the β-chloroalanine derivative (81) in moderate yield as well as the parent amide (40a).



(40a)



(73)



(81)

The relative rates of reaction of the valine derivative (29a), and it s deuterated analogues (29b) and (29c), with NBS were determined in competitive experiments. It was possible to measure the relative rates of reaction of (29a-c) using a mixture of the (2R)-valine derivative (29a) and the (2S)-valine derivative (29b), and of (2R)-(29a) and (2S)-(29c). These mixtures were treated with NBS and heated in refluxing carbon tetrachloride under nitrogen with photochemical initiation. *t.*-Butylbenzamide was used as an internal standard to determine the extent of reaction of the valine derivatives (29a-c). The chirality of the valine derivatives (29a-c) was exploited in the analysis of the mixtures as described in Chapter 1. The starting and product mixtures were analysed by g.l.c. and the initial and final ratios of the valine derivatives (29a-c) measured. The relative rates of reaction of (29a-c) were calculated using equation (14). Whereas the β-

deuterated valine derivative (29b) reacted at the same rate as the unlabelled compound (29a), the α-deuterated valine derivative (29c) reacted 0.27 times as fast as the unlabelled compound (29a) (Table 4). These results indicated a deuterium isotope effect for α-carbon-hydrogen bond homolysis, but no deuterium isotope effect for β-carbon-hydrogen bond homolysis.

The relative rates of reaction of the valine derivatives (29a-c) with di-*t*-butyl peroxide were measured in competitive experiments as described above. Thus, mixtures of the valine derivatives (2R)-(29a) and (2S)-(29b), and of (2R)-(29a) and (2S)-(29c), in benzene were treated with di-*t*-butylperoxide and heated at reflux under nitrogen with irradiation. *t*-Butylbenzamide was used as an internal standard to measure the extent of reaction. The starting and product mixtures were analysed by g.l.c. and the initial and final ratios of the valine derivatives (29a-c) measured. The relative rates of reaction were calculated using equation (14). The α-deuterated valine derivative (29c) reacted 0.67 times as fast as the unlabelled compound (29a) and the β-deuterated valine derivative (29b) reacted 0.53 times as fast as the unlabelled compound (29a). Thus for reaction of the valine derivatives (29a-c) with di-*t*-butyl peroxide there is a deuterium isotope effect for reaction at the α- and β-positions.

Table 4. Relative rates of reaction of the valine derivatives (29a-c).

	Valine Derivative.		
	(29a)	(29b)	(29c)
Reagent			
SO_2Cl_2	1.0*	0.80 ± 0.04	1.00 ± 0.03
DtBP	1.0*	0.53 ± 0.1	0.67 ± 0.04
NBS	1.0*	1.00 ± 0.03	0.27 ± 0.05

*Assigned as unity.

The relative rates of reaction of the (2R)-alanine derivative (40a) and the

(2S)-[2-^{2}H]-alanine derivative (40b) with sulphuryl chloride were measured in competitive experiments as described above. The deuterated alanine derivative (40b) reacted 0.86 times as fast as the unlabelled compound (40a) (Table 5). The relative rates of reaction of the alanine derivative (40a) and it's deuterated analogue (40b) with sulphuryl chloride indicate that there is a small deuterium isotope effect for reaction at the α-position. The relative rates of reaction of the (2R)-alanine derivative (40a) and it's deuterated analogue (2S)-(40b) with NBS were measured in competitive experiments as described above. The deuterated alanine derivative (40b) reacted 0.53 times as fast as the unlabelled compound (40a). The relative rates of reaction of (40a) and (40b) with NBS indicate a deuterium isotope effect for reaction at the α-position.

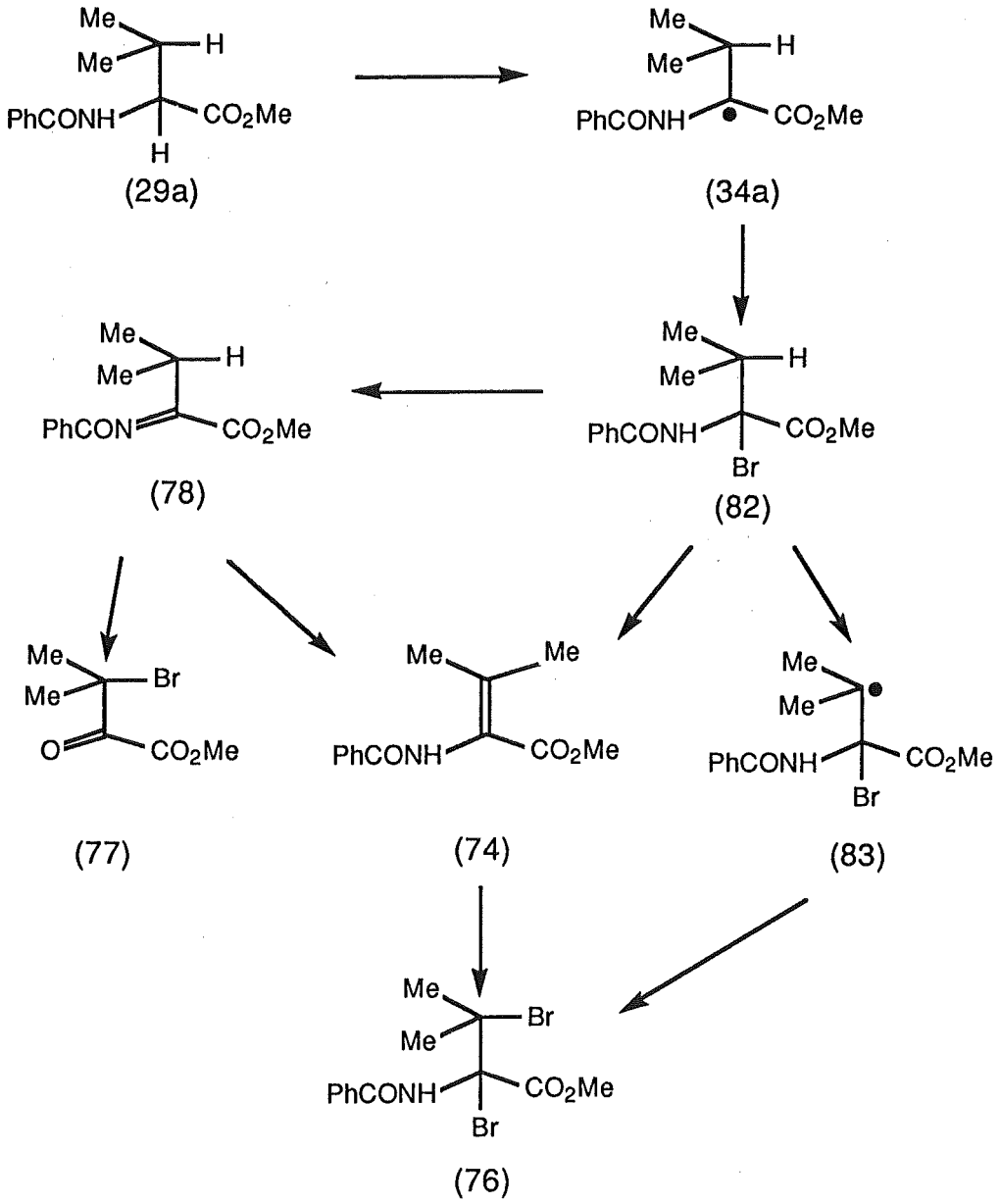
Table 5. Relative rates of reaction of the alanine derivatives (40a) and (40b).

	Alanine Derivative	
Reagent	(40a)	(40b)
SO_2Cl_2	1.0*	0.86 ± 0.05
NBS	1.0*	0.53 ± 0.04

*Assigned as unity.

A solution of the dichlorovaline derivative (75) was treated with 0.9 equivalents of tri-*n*-butyltin hydride in benzene and let stand at room temperature under nitrogen. Chromatography of the concentrated solution afforded the β-chlorovaline derivative (30a). Analysis of crude reaction mixtures by h.p.l.c. showed that the ratio of β-chlorovaline derivative (30a) to valine derivative (29a) produced in these reactions was greater than 100:1.

Two mechanisms were considered to account for the formation of the dibromovaline derivative (76) in the reaction of (29a) with NBS (Scheme 27). That the reaction occurs*via* α-carbon-hydrogen bond homolysis was established

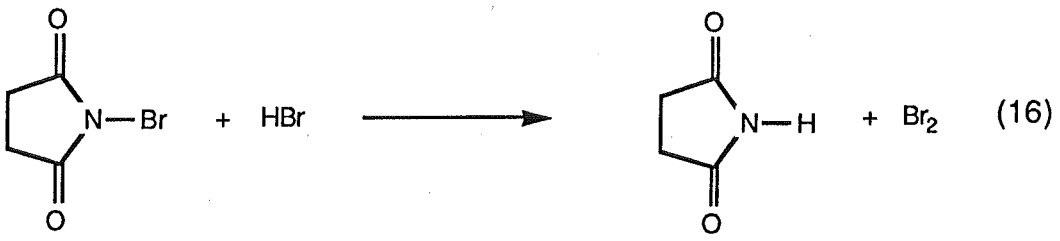


Scheme 27.

by the measurement of a deuterium isotope effect for reaction of the α-deuterated valine derivative (29c) with NBS. One mechanism that accounts for the formation of (76) involves abstraction of the α-hydrogen atom from (29a) to give the α-centred radical (34a) which then incorporates a bromine atom to give the α-bromovaline derivative (82). Further reaction to give the dibromovaline derivative (76), *via* the radical (83), would be expected to be a facile process. It has been observed that in free radical brominations, the position vicinal to a

bromo-substituent undergoes substitution readily.[76] This activation to β-substitution has been ascribed to the formation of a bridged radical intermediate.

The other possible mechanism to account for the formation of (76) also involves formation of the α-centred radical (34a) with subsequent bromine atom incorporation to give the α-bromovaline derivative (82). Elimination of HBr would yield the acylimine (78) which would hydrolyse in the presence of HBr and react with NBS or bromine to give the β-bromo-α-ketoester (77).When excess NBS is present it would act as a scavenging agent to remove HBr rapidly and efficiently (Equation 16).[77] In the absence of HBr reaction of the acylimine (78)

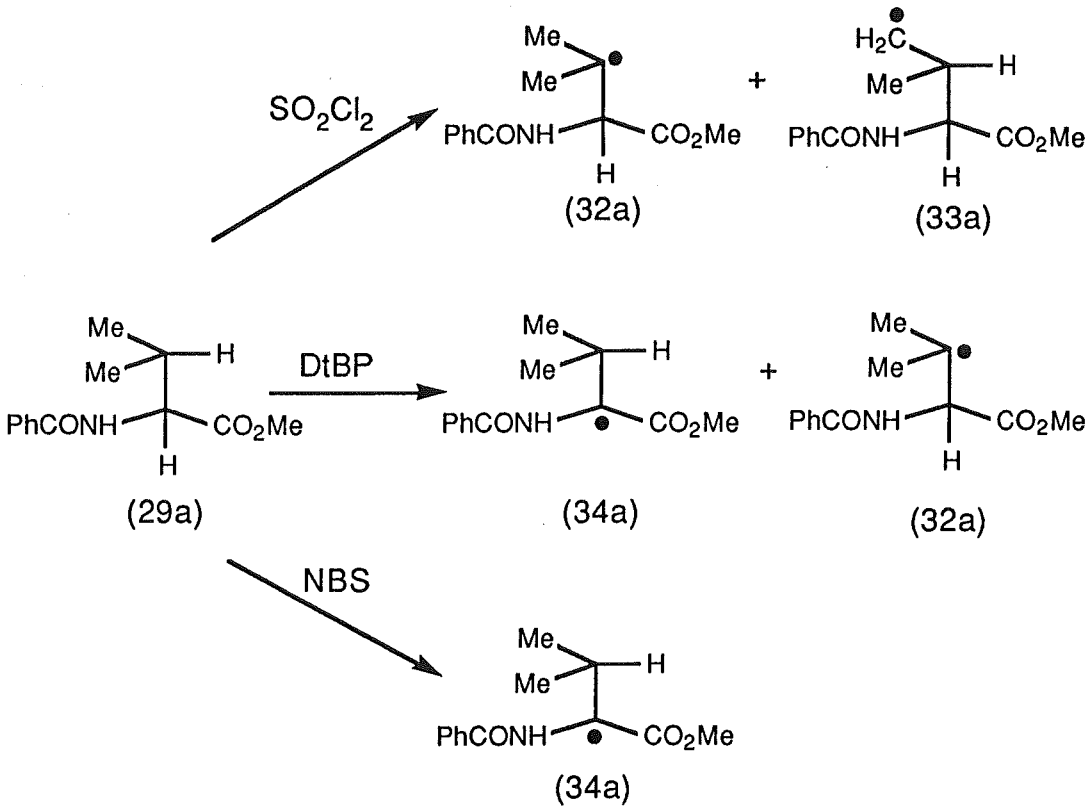


(16)

to give (77) would not occur and (78) would tautomerise to the acylenamine (74), which would react by the addition of bromine to give the dibromovaline derivative (76). The reaction of the acylenamine (74) with two equivalents of NBS to give the dibromovaline derivative (76) is consistent with the latter mechanism. Evidence for the presence of (78) and (74) in reaction mixtures is consistent with both mechanisms. They may be considered as reaction intermediates by the latter mechanism, or by-products of the reaction resulting from HBr elimination from (82) by the former mechanism.

The deuterium isotope studies indicated that reaction of the valine derivative (29a) with NBS occurs *via* α-carbon-hydrogen bond homolysis. This is in direct contrast to the reaction of the valine derivative (29a) with sulphuryl chloride. Whereas the reaction with sulphuryl chloride proceeds *via* β-carbon-hydrogen bond homolysis with formation of the β-centred radical (32a), the

reaction of the valine derivative (29a) with NBS proceeds *via* α-carbon-hydrogen bond homolysis with formation of the α-centred radical (34a). In the reaction of the valine derivative (29a) with di-*t*-butylperoxide there is a deuterium isotope effect for both α- and β-carbon-hydrogen bond homolysis. Hence reaction occurs at either the α- or β-position to form either the α-centred radical (34a) or the β-centred radical (32a), respectively (Scheme 28).



Scheme 28.

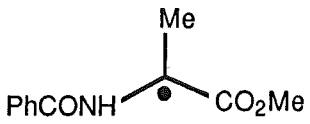
The reversal in selectivity observed in the reactions of the valine derivative (29a) with sulphuryl chloride and NBS may be interpreted in terms of the relative degrees of carbon-hydrogen bond homolysis in the reaction transition states.[60] In hydrogen atom transfer reactions generally, less reactive abstracting radicals react with a greater degree of carbon-hydrogen bond homolysis in the transition state of the reaction, than more reactive radicals. The greater degree of carbon-hydrogen bond homolysis in the transition state of the reactions of less reactive

radicals gives rise to larger deuterium isotope effects in the reactions of these radicals. Free radical chlorination with sulphuryl chloride involves both the chlorosulphonyl radical and the chlorine radical which may be complexed with an aromatic solvent.[38,71,72] The reaction with NBS involves the bromine radical as the abstracting radical[37] and this radical is less reactive than the chlorosulphonyl, chlorine or complexed chlorine radical. Therefore a greater degree of carbon-hydrogen bond homolysis is expected in the transition state of the reaction of the valine derivative (29a) with NBS, than in the transition state of the reaction of (29a) with sulphuryl chloride. The larger deuterium isotope effect observed in the reaction of the valine derivative (29a) with NBS shows that the reaction occurs with a greater degree of carbon-hydrogen bond homolysis in the transition state of the reaction. Thus the reaction is more sensitive to radical stability effects. Hydrogen atom transfer from the α-position is favoured because the product radical (34a) is stabilised by the combined effect of resonance electron-donating amido and electron-withdrawing carboxy groups.

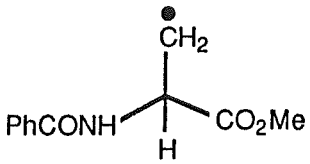
The chlorination reaction occurs with relatively little carbon-hydrogen bond homolysis and, therefore, little development of radical character in the transition state of the reaction. Thus the regioselectivity is controlled by the inductive electron-withdrawing effect of the amido and carboxy substituents. These inductive effects act to retard attack at the adjacent α-position by electrophilic radicals involved in the hydrogen atom abstraction, thus favouring reaction at the β-position.

In the reaction of the valine derivative (29a) with di-*t*-butyl peroxide the extent of bond homolysis in the transition state is intermediate to that found in the chlorination and bromination reactions because the reactivity of *t*-butoxy radicals is intermediate to that of bromine and complexed chlorine radicals.[77] Hence polar effects retarding carbon-hydrogen bond homolysis at the α-position, thus effecting carbon-hydrogen bond homolysis at the β-position, are balanced by resonance effects promoting formation of the α-centred radical (34a). Therefore

reaction occurs at the β-position in competition with reaction at the α-position. The extent to which polar effects outweigh thermodynamic effects in hydrogen atom transfer reactions of amino acid derivatives was examined by investigating reactions of the alanine derivative (40a), and it's deuterated analogue (40b), with NBS and sulphuryl chloride. The relative rates of reaction of the alanine derivatives (40a) and (40b) with sulphuryl chloride show a small deuterium isotope effect for reaction at the α-position. Thus reaction of the alanine derivative (40a) may be assumed to occur, in part, at the α-position to give the radical (84). Given that free radical chlorinations with sulphuryl chloride proceed by hydrogen atom abstraction followed by chlorine atom incorporation at the site of hydrogen atom abstraction,[38] formation of the β-chloroalanine derivative (81) shows that the reaction of the alanine derivative (40a) with sulphuryl chloride proceeds, in part, by formation of the primary radical (85). That the photolysis of the N-chloroalanine derivative (73) affords the β-chloroalanine derivative (81) is further evidence for formation of the radical (85).



(84)



(85)

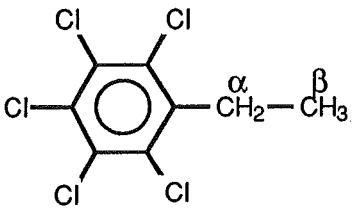
The selectivity observed in the chlorination of the alanine derivative (40a) illustrates the balance between inductive and radical stability effects operating in the formation of the α-centred tertiary radical (84) and the β-centred primary radical (85). Formation of tertiary radicals is thermodynamically favoured over formation of primary radicals but in this case the expected greater stability of the tertiary amidocarboxy-substituted radical (84) is balanced by the destabilisation of the transition state afforded by the inductively electron-withdrawing amido and carboxy substituents. Thus formation of the primary radical (85) competes with

formation of the tertiary radical (84).

The relative rates of reaction of the alanine derivatives (40a) and (40b) with NBS indicate that reaction proceeds *via* α-carbon-hydrogen bond homolysis. Reaction at the α-position occurs because extensive bond homolysis in the transition state of the bromination ensures the stabilising resonance effects of the amido and carboxy substituents are dominant.

Similar reversals in selectivity have been observed in a number of hydrogen atom transfer reactions before and have been explained in terms of the degree of bond homolysis in the transition of the reactions. Russell and Brown[78] observed that toluene is 250 times more reactive than cyclohexane, on a per hydrogen basis, towards bromination, but 3.8 times less reactive towards chlorination. The free radical halogenation of toluene proceeds *via* the resonance stabilised benzyl radical and the free radical halogenation of cyclohexane proceeds *via* cyclohexyl radical which is less stable than benzyl radical. The bromination of the compounds occurs with greater bond homolysis in the transition states of the reactions than the chlorination of the compounds because of the greater selectivity of the bromine atom relative to the chlorine atom. Thus radical stability effects predominate and the bromination of toluene is favoured over the bromination of cyclohexane because the benzyl radical is more stable than the cyclohexyl radical. The chlorination of the compounds, which occurs with less bond homolysis in the transition states, is less affected by radical stability effects and inductive effects predominate. The phenyl group is inductively electron-withdrawing, relative to an alkyl group, hence it is destabilising to an electrophilic radical such as a chlorine atom. Thus the chlorination of toluene is retarded relative to the chlorination of cyclohexane by electron withdrawing inductive effects.

A reversal of selectivity has been observed in the free radical halogenation of 1-ethylpentachlorobenzene (86) and has also been explained in terms of the degree of bond homolysis in the reaction transition states.[79]



(86)

Bromination of (86) occurred at the α-position whereas the chlorination occurred at the β-position. Bromination at the α-position may be attributed to attack by the bromine radical proceeding with extensive development of radical character in the transition state, thus resonance electron-donating effects favour formation of the radical at the α-position. Chlorination at the β-position may be attributed to attack by the chlorine atom proceeding with little development of radical character in the transition state, thus inductive electron-withdrawing effects retard reaction at the α-position.

These observations add further support for the rationale that the selectivity in the reaction of the valine derivative (29a) with abstracting radicals is controlled by the degree of bond homolysis in the transition state of the reaction.

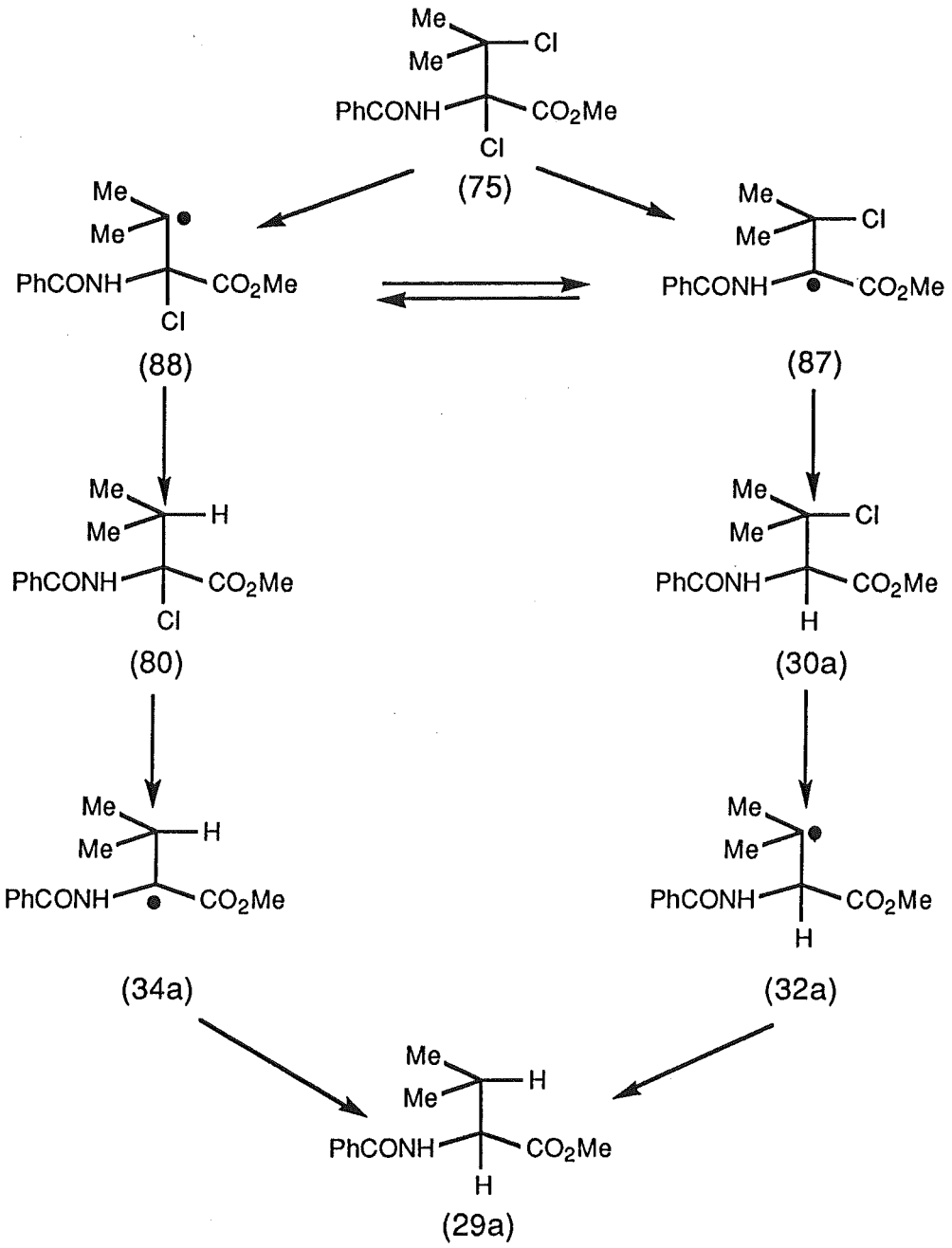
The reaction of the dichloride (47) with tri-*n* -butyltin hydride was examined to study halogen atom transfer from a valine derivative. Reductions of alkyl chlorides by organotin hydrides are thought to proceed *via* a free radical mechanism (equations 17-22).[80] The chain carrying steps of the proposed mechanism are equations 18 and 19.

$R'_3SnH + Q^{\bullet}$	$\rightarrow$	$R'_3Sn^{\bullet} + QH$	(17)
$R'_3Sn^{\bullet} + RX$	$\rightarrow$	$R^{\bullet} + R'_3SnX$	(18)
$R^{\bullet} + R'_3SnH$	$\rightarrow$	$RH + R'_3Sn^{\bullet}$	(19)
$2R'_3Sn^{\bullet}$	$\rightarrow$	$(R'_3Sn)_2$	(20)
$R^{\bullet} + R'_3Sn^{\bullet}$	$\rightarrow$	R'_3SnR	(21)
$2R^{\bullet}$	$\rightarrow$	R(+H) + R(-H) or R-R	(22)

The relative ease of halogen atom abstraction by an organotin radical may be determined from reactions of this type. A halide may be placed in competition with another for an insufficient amount of hydride and the product ratio indicates the relative ease of halogen atom abstraction by the organotin radical. Reaction of the dichloride (75) with tri-*n* -butyltin hydride provides a choice of two chlorine atom abstractions. Assuming that the stability of the free radical intermediate is a prime factor in determining the rate of halogen atom abstraction[81,82] the product ratio mirrors the relative stabilities of the intermediate radicals. In the unlikely event that a chlorine atom abstraction from the vicinal dichloride (75) affords the less stable of the possible intermediate radicals, a facile 1,2-chlorine atom migration to give the more thermodynamically stable radical would be expected.[83] This radical would incorporate a hydrogen atom to give the mono-chlorinated product.

Formation of the β-chlorovaline derivative (30a) shows that the α-centred radical (87) is more stable than the β-centred radical (88). Further, the production of only trace amounts of the valine derivative (29a), the product of the subsequent reduction of (30a), in the reaction with tri-*n* -butyltin hydride shows that the radical (87) is more stable than (32a). From this study the inference may be drawn that the amidocarboxy-substituted radical (34a) is more stable than the tertiary radical (32a).

These results support the conclusion that the difference in the reactions of N-benzoylvaline methyl ester (29a) with sulphuryl chloride and NBS can be attributed to the relative degree of carbon-hydrogen bond homolysis in the respective transition states of the reactions. Extensive bond homolysis and the development of radical character in the transition state of the reaction of (29a) with NBS results in reaction *via* α-carbon-hydrogen bond homolysis to give the amidocarboxy-substituted radical (34a). Conversly, the lack of development of radical character in the transition state of the reaction of (29a) with sulphuryl



Scheme 29.

chloride is evident in regioselectivity determined by inductive effects and results in β-carbon-hydrogen bond homolysis to give the radical (32a). The work presented in this chapter shows that amidocarboxy-substituted radicals such as (34a) are considerably more stable than, for example, the corresponding tertiary alkyl radical (32a) but hydrogen-atom transfer reactions may afford less stable

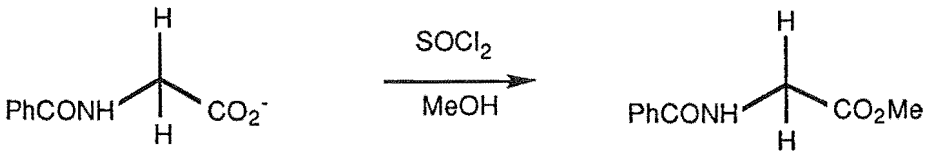
products if electrophilic radicals are involved in the hydrogen atom abstraction and if there is little development of radical character in the transition state. Interpretation of the work in terms of the development of radical character in the transition state of the reaction rationalises the apparent discord between the reactions of (23) to give (25) and (29a) to give (30a). Analogously regioselective hydrogen atom transfer reactions such as (5) to give the corresponding radical (12) in penicillin biosynthesis, and (19) to give (22) in the β-hydroxylation of valine residues have been established as chemically valid.

CHAPTER 3

PREFERENTIAL REACTIVITY OF GLYCINE RESIDUES IN FREE RADICAL REACTIONS.

Reactions of derivatives of valine (29a), alanine (40a), glycine (93a), pyroglutamic acid (23a) and proline (100a), and their deuterated analogues (29c), (40b), (93b), (23b), (100b) and (100c), were studied to examine the previously unexplained reactivity of glycine residues in free radical reactions of amino acid derivatives.

N-benzoylglycine methyl ester (93a) was prepared by dissolving hippuric acid (45) in methanol to which thionyl chloride had been added, and standing overnight (Scheme 30). Removal of the solvent and chromatography of the residue gave the glycine derivative (93a).

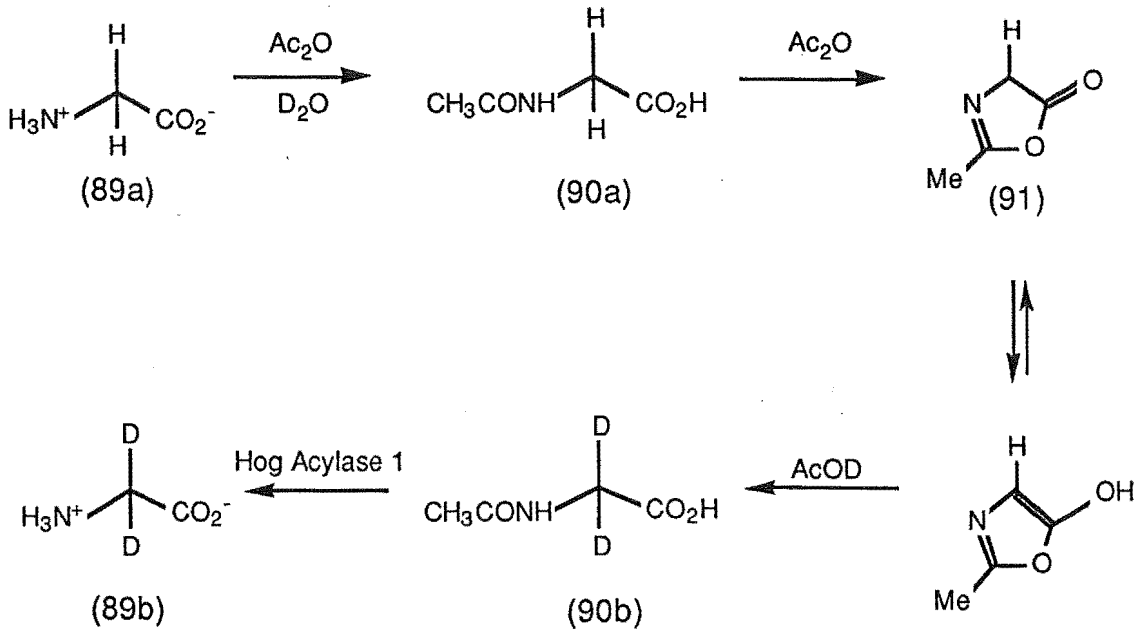


(45) (93a)

Scheme 30.

[2,2-$^2H_2$]-Glycine (90b) was prepared from glycine (90a) using the method of Greenstein and Winitz (Scheme 31).[63] Incorporation of deuterium at the α-position was accomplished through isomerisation of the azlactone (91), formed by treatment of glycine (89a) with more than two equivalents of acetic anhydride in acetic acid-OD, and hydrolysis of the resultant N-acetyl derivative (90b). Hence, glycine (89a) was treated with D_2O in acetic anhydride and heated at reflux, under nitrogen. More D_2O was added, the solution cooled and the solvent removed *in vacuo* . The crude N-acetyl-[2,2-$^2H_2$]-glycine (90b) was heated at

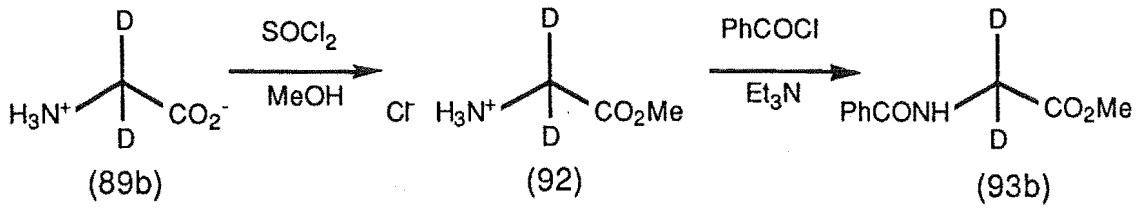
reflux in 6M HCl, the solvent removed and [2,2-$^2H_2$]-glycine (89b) crystallised from ethanol / aniline.



Scheme 3 1.

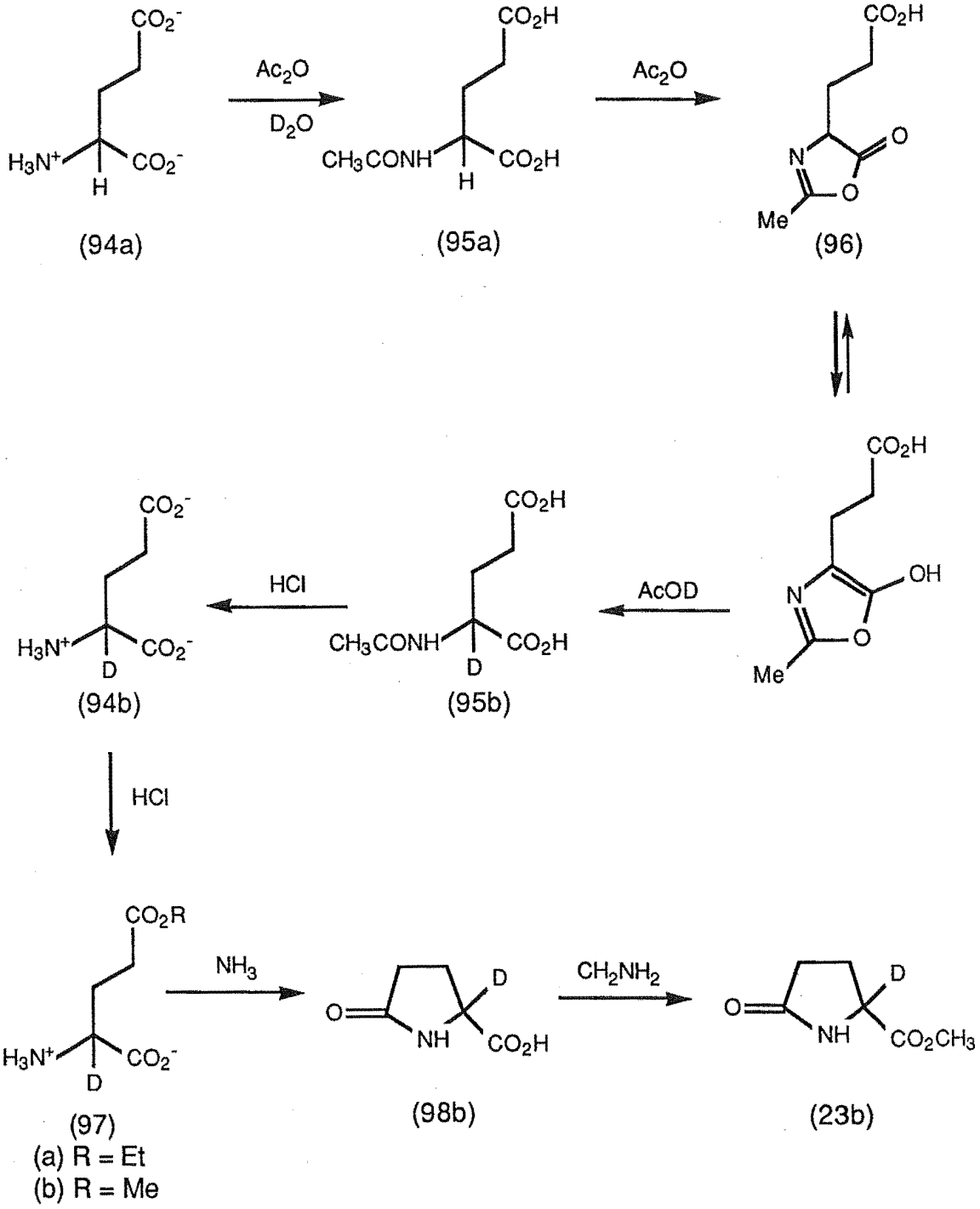
N-Benzoyl-[2,2-$^2H_2$]-glycine methyl ester (93b) was prepared from [2,2-$^2H_2$]-glycine (89b) using the method of Applewhite *et.al.* [64] as described below (Scheme 32). [2,2-$^2H_2$]-Glycine (89b) was dissolved in methanol to which thionyl chloride had been added and left to stand for 16 hours. The solvent was removed *in vacuo* and the crude [2,2-$^2H_2$]-glycine methyl ester hydrochloride (92) was dissolved in ethyl acetate and treated with triethylamine and benzoyl chloride. Work up and chromatography afforded the deuterated glycine derivative (93b).

Methyl pyroglutamate (23a) was synthesised from pyroglutamic acid (98a). The acid (98a) was dissolved in methanol and treated with a solution of diazomethane in ether and left to stand for 16 hours. The solvent was removed and purification of the residue using a Kugelrohr apparatus afforded methyl pyroglutamate (23a).



Scheme 32.

Methyl [2-^{2}H]-pyroglutamate (23b) was synthesised from glutamic acid (94a) (Scheme 33). Deuterium was incorporated at the α-position of glutamic acid (94a) by isomerisation of the azlactone (96), formed by treatment of glutamic acid (94a) with more than two equivalents of acetic anhydride in acetic acid-OD, and hydrolysis of the resultant N-acetyl [2-^{2}H]-glutamic acid derivative (95b).[63] The [2-^{2}H]-glutamic acid (94b) was esterified at the γ-position, cyclised to give [2-^{2}H]-pyroglutamic acid (98b),[84] and derivatised to give methyl-[2-^{2}H]-pyroglutamate (23b). Hence, glutamic acid (94a) was heated at reflux with D_2O in acetic anhydride. More D_2O was added, the solution cooled and the solvent removed *in vacuo*. The crude N-acetyl-[2-^{2}H]-glutamic acid (95b) was heated at reflux in 6M HCl, the solvent removed and the crude [2-^{2}H]-glutamic acid (94b) dissolved in methanol in which an equivalent of dry HCl gas had been dissolved. After standing the solvent was removed, the residue washed with methanol and neutralised with ammonium hydroxide. Crystallisation from ethanol afforded the γ-ethyl-[2-^{2}H]-glutamic acid (97a) rather than the expected γ-methyl-[2-^{2}H]-glutamic acid (97b), presumably the result of trans-esterification during crystallisation. The difference in structure was deduced from the melting point[84] and the ^{13}C n.m.r. spectrum of the sample. The ester (97a) was dissolved in methanol saturated with ammonia and left to stand overnight. The solvent was removed and the residue treated with a solution of diazomethane in ether. Removal of the solvent and purification using a Kugelrohr apparatus afforded

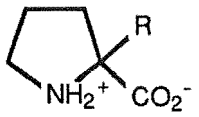


Scheme 33.

methyl-[2-^{2}H]-pyroglutamate (23b).

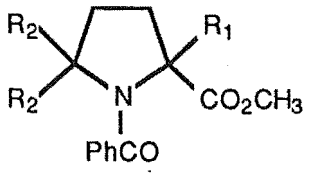
[2-^{2}H]-Proline (99b) was prepared from proline (99a) using the method of Greenstein and Winitz[63] as described above. The proline derivative (100a) and

it's deuterated analogue (100b) were prepared from their respective amino acids using the method of Applewhite *et. al.*[64] as described above.



(99)

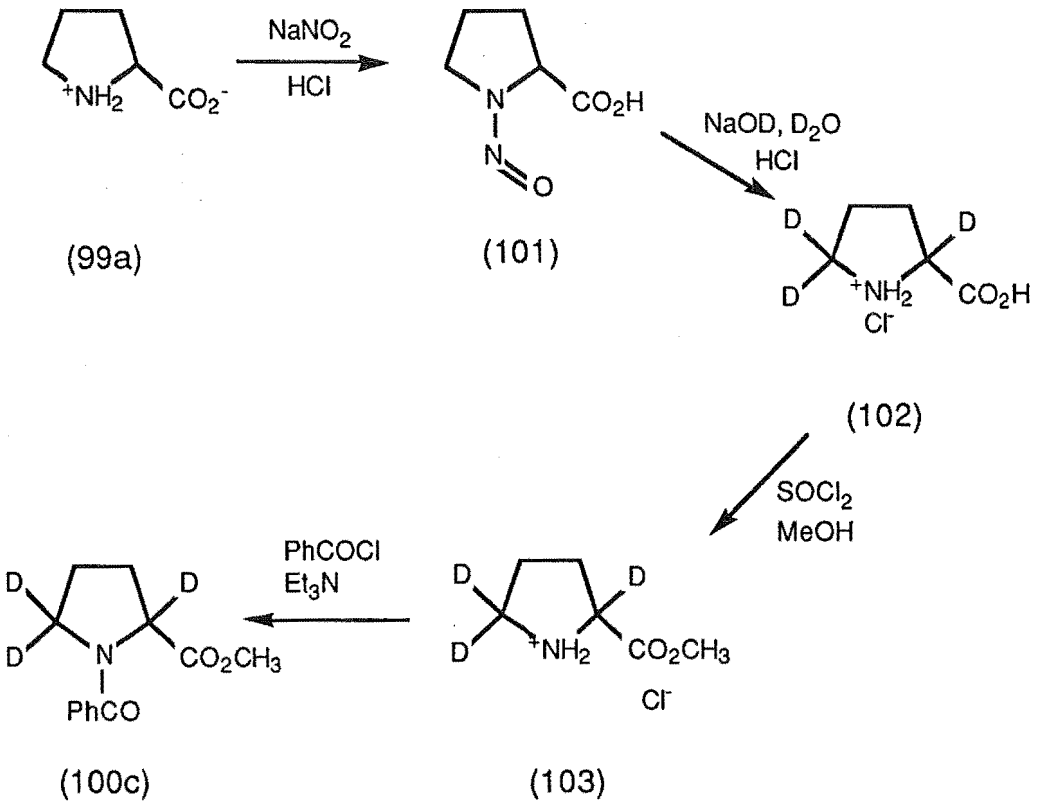
(a) R = H
(b) R = D



(100)

(a) $R_1 = R_2 = H$
(b) $R_1 = D$, $R_2 = H$
(c) $R_1 = R_2 = D$

The trideuterated derivative (100c) was synthesised using methods of Leitch,[85] Keefer and Fodor,[86] Lijinsky *et. al.*,[87] and Applewhite *et. al.*[64] (Scheme 34). Sodium nitrite was added to a cooled acidic solution of (2S)-

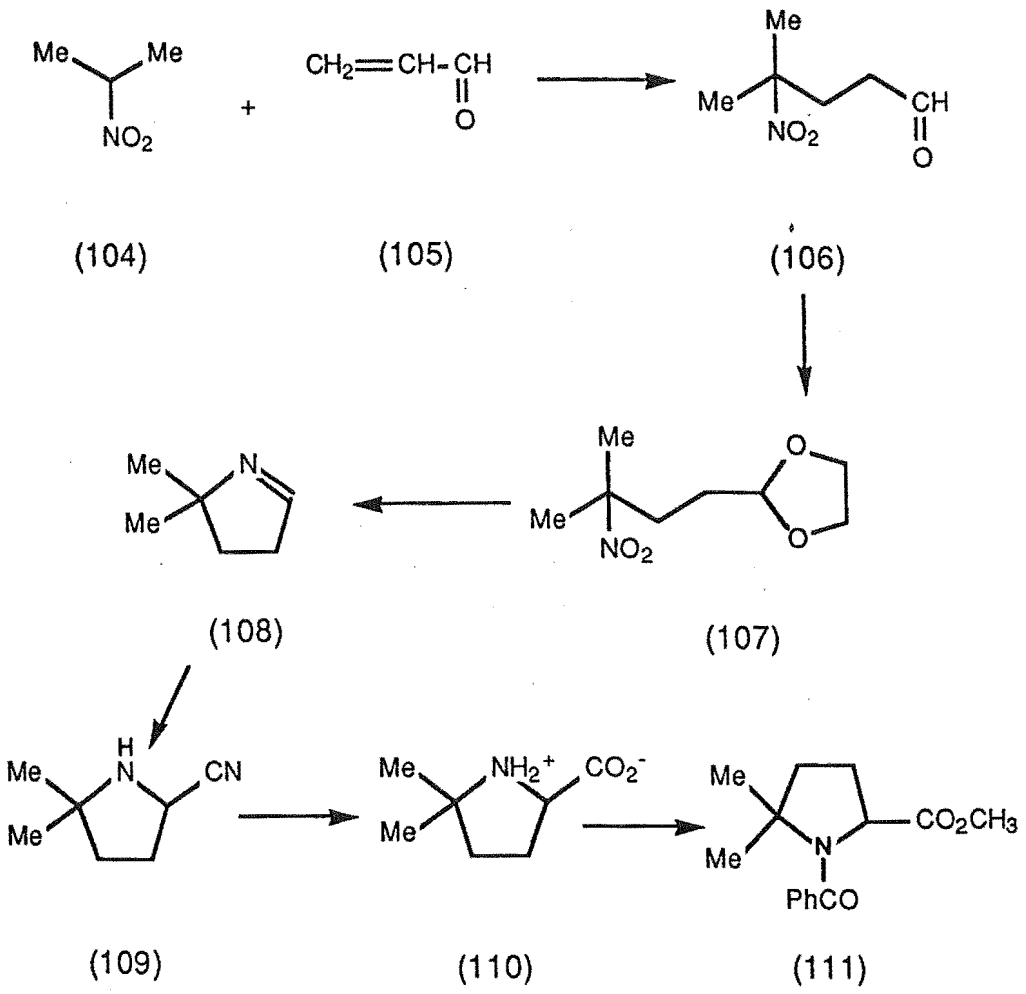


(99a) (101) (102) (103) (100c)

Scheme 34.

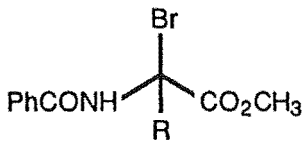
proline (100a) and left to stand for 90 minutes. The solvent was removed and N-nitrosoproline (101) extracted with acetone. The recrystallised N-nitrosoproline (101) was heated at reflux with sodium deuteroxide in D_2O, *c* HCl was added and the solution heated at reflux for 8 hours. The solvent was removed and the crude [2,5,5-$^2H_3$]-proline hydrochloride (102) was dissolved in methanol to which thionyl chloride had been added, and left to stand for 16 hours. The solvent was removed and the crude [2,5,5-$^2H_3$]-proline methyl ester hydrochloride (103) dissolved in ethyl acetate and treated with triethylamine and benzoyl chloride. Work up and chromatography afforded the trideuterated proline derivative (100c).

N-Benzoyl 5,5-dimethylproline methyl ester (111) was prepared according to the method of Bonnett *et. al.*[88] (Scheme 35). 4-Methyl-4-nitropentan-1-al (106) was prepared by the condensation of acrolein (104) and 2-nitropropane (105) in the presence of sodium methoxide. 4-Methyl-4-nitropentan-1-al (106), dry ethylene glycol and toluene-*p* -sulphonic acid in benzene were heated at reflux in a Dean-Stark apparatus. Work up of the benzene solution and distillation afforded 2-(3-methyl-3-nitrobutyl)-1,3-dioxolan (107). The dioxolan (107) was hydrogenated over Raney nickel at room temperature and 1800 p.s.i. for twenty-four hours. The catalyst and solvent were removed and the residue heated at reflux in 6M HCl. Work up gave the crude 5,5-dimethyl-Δ^1-pyrroline (108) which was treated with KCN, left to stand overnight, treated with base and extracted into ether. Evaporation of the solvent gave the crude 5-cyano-2,2-dimethylpyrrolidine (109) which was hydrolysed to give the crude 5,5-dimethyl-proline hydrochloride (110). Derivatisation of (110) as described above afforded N-benzoyl-5,5-dimethylproline methyl ester (111).



Scheme 35.

The glycine derivative (93a) was treated with one equivalent of NBS in refluxing carbon tetrachloride, under nitrogen, with irradiation by a 250 W UV lamp. The solution was cooled and filtered to remove succinimide and any remaining NBS and the solvent removed *in vacuo* . This reaction of the glycine derivative (93a) with NBS gave the α-bromoglycine derivative (112a)[89] in high yield. There was no evidence of the formation of the dibromoglycine derivative (112b). Assignment of the structure of (112a) was made on the basis of the ^1H n.m.r. spectrum. The presence of a doublet for the α-proton at δ 6.56 ppm (J = 8 Hz) is consistent with the α-bromoglycine derivative (112a).

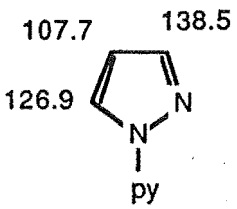


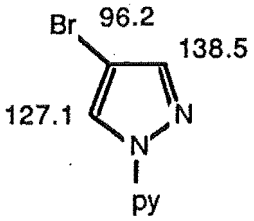
(112)
(a) R = H
(b) R = Br

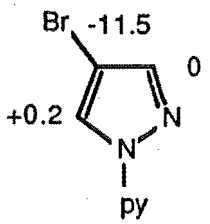
Treatment of methyl pyroglutamate (23a) with NBS in refluxing carbon tetrachloride, under nitrogen, with irradiation by a 250 W UV lamp, gave a gummy red oil, which could not be resolved using numerous chromatographic systems.

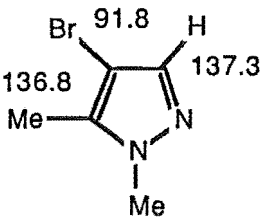
Reaction of the proline derivative (100a) with one equivalent of NBS in refluxing carbon tetrachloride, as described above, failed to give complete reaction as shown by h.p.l.c. analysis. Reaction with three equivalents of NBS ensured complete reaction of the proline derivative (100a) and afforded N-benzoyl-2-methoxycarbonyl-4-bromopyrrole (113) and N-benzoyl-2-methoxy-carbonyl-3-bromopyrrole (114). Also observed in the reaction mixture was N-benzoyl-2-methoxycarbonylpyrrole (115), presumably as a result of incomplete reaction. Mass spectra of (113) and (114) confirmed the compounds as mono-brominated pyrrole derivatives. Both compounds gave two parent ions at *m/z* 309 and 307. The structures of the isomers of the monobromopyrroles (113) and (114) were deduced by comparison of the ^{1}H and ^{13}C n.m.r. spectra with those of the unsubstituted pyrrole (115). The expected effects of monobromination at the three possible ring positions on the ^{13}C n.m.r. chemical shifts of the ring carbon atoms were calculated by comparison of the variously substituted pyr azoles (Table 6).[90] These calculated values were correlated with the observed values to make the assignments (Table 7). The chemical shifts of the ring protons in the ^{1}H n.m.r. spectra also supported the assignments (Table 8).

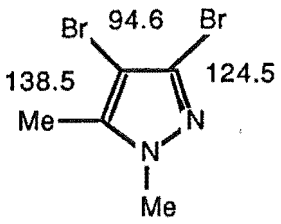
Table 6. ^{13}C N.m.r chemical shifts (ppm) for ring carbons of substituted pyr azoles.

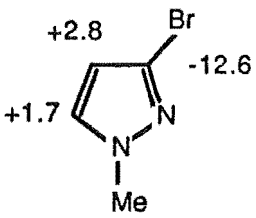


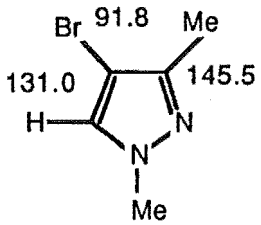


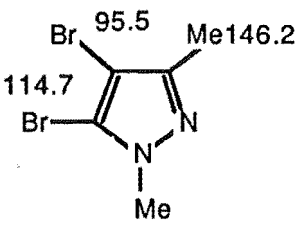


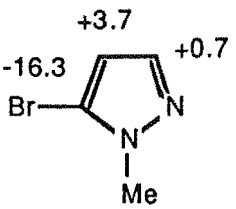


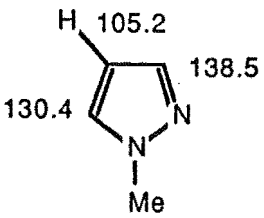


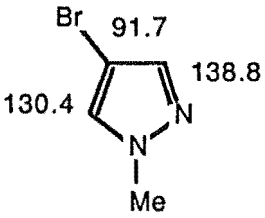


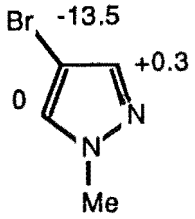




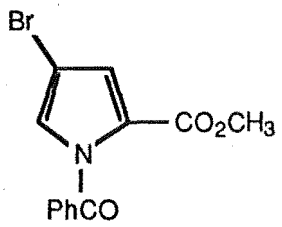




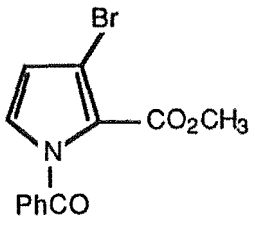




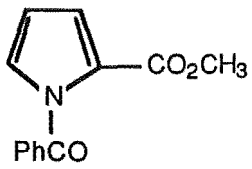
*py = 2-pyridyl



(113)



(114)



(115)

Table 7. ^{13}C N.m.r. chemical shifts (ppm) for ring carbons of pyrroles (114)-(116).

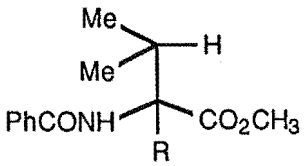
Compound		Ring Position		
		3	4	5
(115)	obs.	121.3	110.6	127.7
(114)	calc.	108.7	113.4	129.4
	obs.	109.4	114.9	126.3
(113)	calc.	121.6	97.1	127.7
	obs.	122.5	99.3	126.6

Table 8. ^{1}H N.m.r. chemical shifts (ppm) for pyrroles (114)-(116).

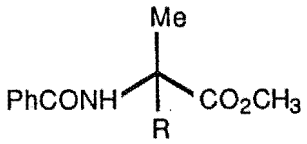
Compound	Ring Position		
	3	4	5
(115)	7.07	6.31	7.23
(114)	---	6.39	7.14
(113)	7.03	---	7.22

Examination of the reaction of the dimethylproline derivative (111) with NBS showed that the dimethylproline derivative (111) reacted with NBS in the absence of UV light. This suggested that reaction of the dimethylproline derivative (111) with NBS did not involve a free radical mechanism and hence (111) was unsuitable for this study.

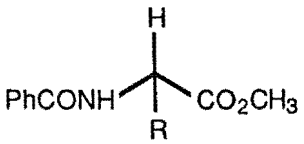
The relative rates of reaction of the amino acid derivatives (29a), (40a) and (93a), and their deuterated analogues (29c), (40b) and (93b), with NBS were determined in competitive experiments. Solutions of the derivatives, for example, N-benzoylalanine methyl ester (40a) and N-benzoylglycine methyl ester (93a) were treated with one equivalent of NBS in refluxing carbon tetrachloride, under nitrogen. The mixtures were irradiated with a 250 W mercury lamp. *t*-Butylbenzamide was used as an internal standard to measure the extent of reaction. The initial and product mixtures were analysed by h.p.l.c. and the initial and final ratios of amino acid derivatives measured. The relative rates of reaction were calculated using equation (14). The relative rates of reaction of the amino acid derivatives are presented in Table 9.



(29)
(a) R = H
(c) R = D



(40)
(a) R = H
(b) R = D



(93)
(a) R = H
(b) R = D

The competitive experiments show that the glycine derivative (93a) reacts faster than the alanine derivative (40a), and that both the glycine derivative (93a) and the alanine derivative (40a) react considerably faster than the valine derivative (29a). The unlabelled valine derivative (29a) reacts 3.7 times as fast

as the deuterated compound (29c). This deuterium isotope effect for reaction at the α-position is in agreement with the result obtained by analysis a g.l.c. column with a chiral stationary phase (Table 4). The unlabelled alanine derivative (40a) reacts 1.9 times as fast as the deuterated compound (40b). This deuterium isotope effect for the reaction at the α-position is also in agreement with the result obtained from analysis by g.l.c. (Table 5). The unlabelled glycine derivative (93a) reacts 3.1 times as fast as the deuterated compound (93b).

Table 9. Relative rates of reaction of the amino acid derivatives with NBS.

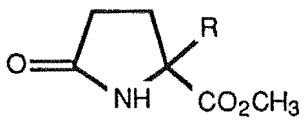
Amino acid derivative.		k_H		k_D
Valine	(29a)	1.0*	(29c)	0.27 ± 0.2
Alanine	(40a)	7.7 ± 0.2	(40b)	4.1 ± 0.4
Glycine	(93a)	23 ± 3.5	(93b)	7.3 ± 0.8
Glycine	(93a)	1.0*	(93b)	0.32 ± 0.03
Proline	(100a)	1.41 ± 0.1	(100b)	1.2 ± 0.1
			(100c)	0.42 ± 0.04
Methyl pyroglutamate	(23a)	3.1 ± 0.7	(23b)	2.1 ± 0.3

*Assigned as unity.

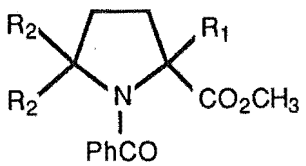
The errors in the analysis by h.p.l.c. are larger than those determined by g.l.c. analysis because the method requires comparison between sets of results, *ie.*, the errors are cumulative. For example, the deuterium isotope effect for the alanine derivative (40) was obtained from two experiments; the rate of reaction of the alanine derivative (40a) relative to the rate of reaction of the glycine

derivative (93a), and the rate of reaction of the deuterated alanine derivative (40b) relative to that of the glycine derivative (93a). Analysis by h.p.l.c. gave a greater variation in results compared to g.l.c. analysis and this was reflected in the errors which encompass the range of experimental values.

The relative rates of reaction of methyl pyroglutamate (23a), and its deuterated analogue (23b), with NBS were measured in competitive experiments. The rates of reaction of (23a) and (23b) were determined relative to the rate of reaction of the glycine derivative (93a). Mixtures of (23a) and (93a), or (23b) and (93a), were treated with NBS in carbon tetrachloride as described above. *t*-Butylbenzamide was used as internal standard. The initial and product mixtures were analysed by g.l.c. using a Carbowax 30M capillary column and the initial and final ratios of the compounds measured. H.p.l.c. was not used to analyse the mixtures containing methyl pyroglutamate (23a), or (23b), because of the poor response factors for detection by the ultra-violet detector. The relative rates of reaction were calculated using equation (14). Whereas methyl pyroglutamate (23a) reacts 3.1 times as fast as the glycine derivative (93a), the deuterated derivative (23b) reacts only 2.1 times as fast as the glycine derivative (93a). Thus, the reaction of methyl pyroglutamate with NBS exhibits a deuterium isotope effect of 1.5 ± 0.5 for reaction at the α-position. This relatively low value for the deuterium isotope effect may be attributed to the low deuterium incorporation in (23b) (62%).



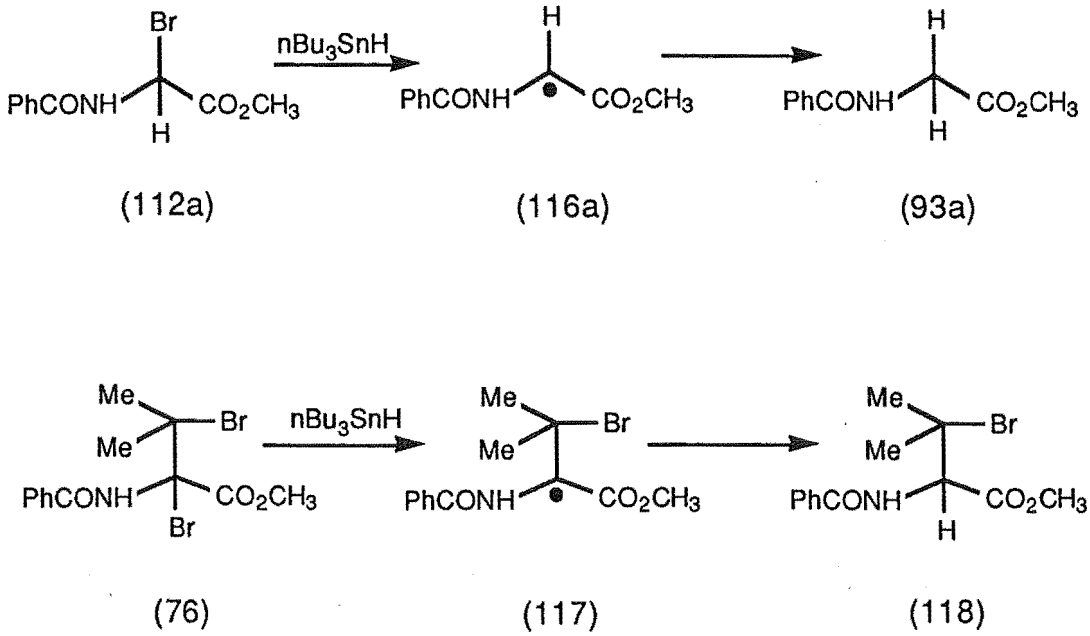
(23)
(a) R = H
(b) R = D



(100)
(a) $R_1 = R_2 = H$
(b) $R_1 = D$, $R_2 = H$
(c) $R_1 = R_2 = D$

The relative rates of reaction of the proline derivative (100a), and its deuterated analogues (100b) and (100c), with NBS, were determined in competitive experiments, with analysis by h.p.l.c., as described above (Table 9). Whereas the proline derivative (100a) reacts 1.41 times as fast as the glycine derivative (93a) the deuterated analogue (100b) reacts 1.2 times as fast as the glycine derivative (93a). The trideuterated proline derivative (100c) reacts 0.42 times as fast as the glycine derivative (93a). This represents a deuterium isotope effect of 1.2 for hydrogen atom abstraction at the α-position and a deuterium isotope effect of 2.85 for hydrogen atom abstraction at the δ-position of the proline derivative (100).

To further investigate the relative stabilities of radicals produced in free radical reactions of amino acid derivatives, competitive reactions of the α-bromoglycine derivative (112a) and the dibromovaline derivative (76) with tri-*n*-butyltin hydride were examined. The α-bromoglycine derivative (112a) was synthesised as described above. The dibromovaline derivative (76) was synthesised as described in Chapter 2. Treatment of the α-bromoglycine derivative (112a) with tri-*n*-butyltin hydride afforded the glycine derivative (93a) (Scheme 36). This was determined by h.p.l.c. analysis and ^{1}H n.m.r. spectroscopy. Reaction of the dibromovaline derivative (76) was shown to afford the β-bromovaline derivative (118) (Scheme 36).[91] Mixtures of the α-bromoglycine derivative (112a) and the dibromovaline derivative (76) were treated with 0.9 equivalents of tri-*n*-butyltin hydride in benzene at room temperature. Analysis by h.p.l.c. and ^{1}H n.m.r. spectroscopy showed the selective reduction of the α-bromoglycine derivative (112a) to give the glycine derivative (93a). There was no evidence of reduction of (76) to give the β-bromovaline derivative (118) in the competitive experiments.

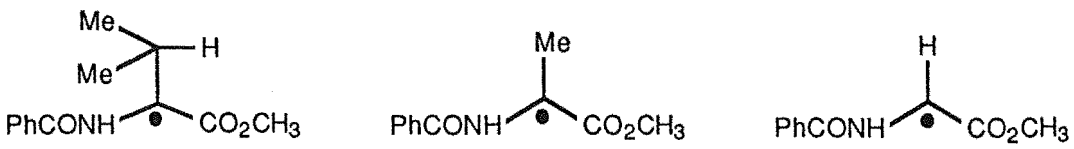


(112a) (116a) (93a)

(76) (117) (118)

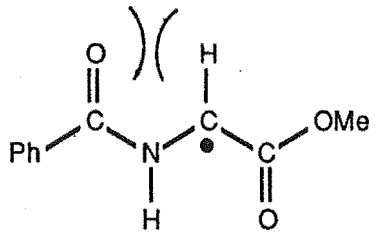
Scheme 36.

The deuterium isotope effects observed in the reactions of (29), (40) and (93) with NBS show that α-carbon hydrogen bond homolysis is the irreversible rate determining step. Having established that the reactions of (29a), (40a) and (93a) proceed *via* α-carbon-hydrogen bond homolysis, the relative rates of reaction of (29a), (40a) and (93a) with NBS reflect the relative ease of α-hydrogen atom abstraction from these compounds. The results show that hydrogen atom abstraction from the glycine derivative (93a) to give the secondary radical (116a) is faster than hydrogen atom abstraction from the

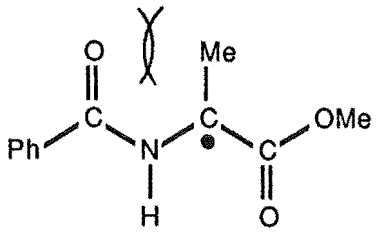


(34a) (84) (116a)

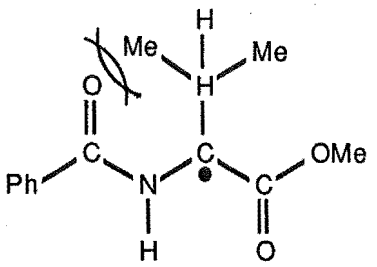
alanine derivative (40a) to give the tertiary radical (84). Hydrogen atom abstraction from the valine derivative (29a) to give the tertiary radical (34a) is considerably slower than either of the hydrogen atom abstractions from the alanine derivative (40a) and the glycine derivative (93a). Thus it appears from the relative rates of reaction of (29a), (40a) and (93a) with NBS that the secondary glycyl radical (116a) is marginally more stable than the tertiary alanyl radical (84), and that both (116a) and (84) are considerably more stable than the tertiary valinyl radical (34a). The relative stabilities of the radicals (116a) and (34a) were further investigated through the reactions of the α-bromoglycine derivative (112a) and the dibromovaline derivative (76) with tri-*n*-butyltin hydride. Reactions of alkyl halides with organotin hydrides have been discussed in Chapter 2. It is thought that the stability of the free radical intermediate is a prime factor in determining the rate of halogen atom abstraction.[81,82] Hence, in competitive experiments, the product ratio mirrors the relative stability of the intermediate radicals. Selective formation of the glycine derivative (93a) in the competitive experiments, shows that the secondary radical (116a) is more stable than the tertiary radical (117). This peculiar stability of the radical (116a) is attributed to a particularly favourable geometry. Stabilisation of the captodative radicals (34a), (84) and (116a) results from overlap of the semi-occupied p-orbitals with the π-orbitals of the amido and carboxy substituents. There is maximum overlap of these orbitals in planar conformations of the radicals (34a), (84) and (116a), such as those shown in Figure 3. The radical (116a) will be destabilised compared to (84) by non-bonding interactions associated with planar conformations of (116a), and (34a) will be even less stable owing to more severe non-bonding interactions. These destabilising influences outweigh the expected thermodynamic preference for the production of the tertiary radicals.[60] The lack of subsequent reaction of the α-bromoglycine derivative (116a) to produce the dibromoglycine derivative (112b) is consistent with the rationale proposed for the selective reactivity of the glycine derivative (93a). Reaction to



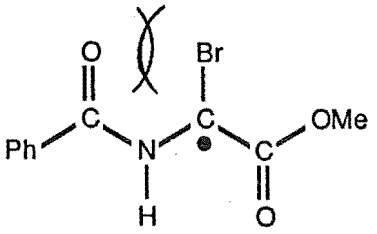
(116a)



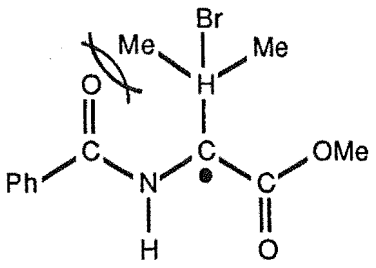
(84)



(34a)



(116b)



(117)

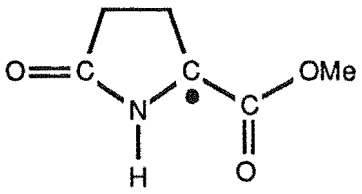
Figure 3.

give dibromoglycine derivative (112b) would involve reaction *via* the α-bromoglycyl radical (116b) which is expected to be less stable than the glycyl radical (116a) because of the nonbonding interaction associated with the relatively large bromine substituent.

That the reaction of mixtures of the α-bromoglycine derivative (116a) and

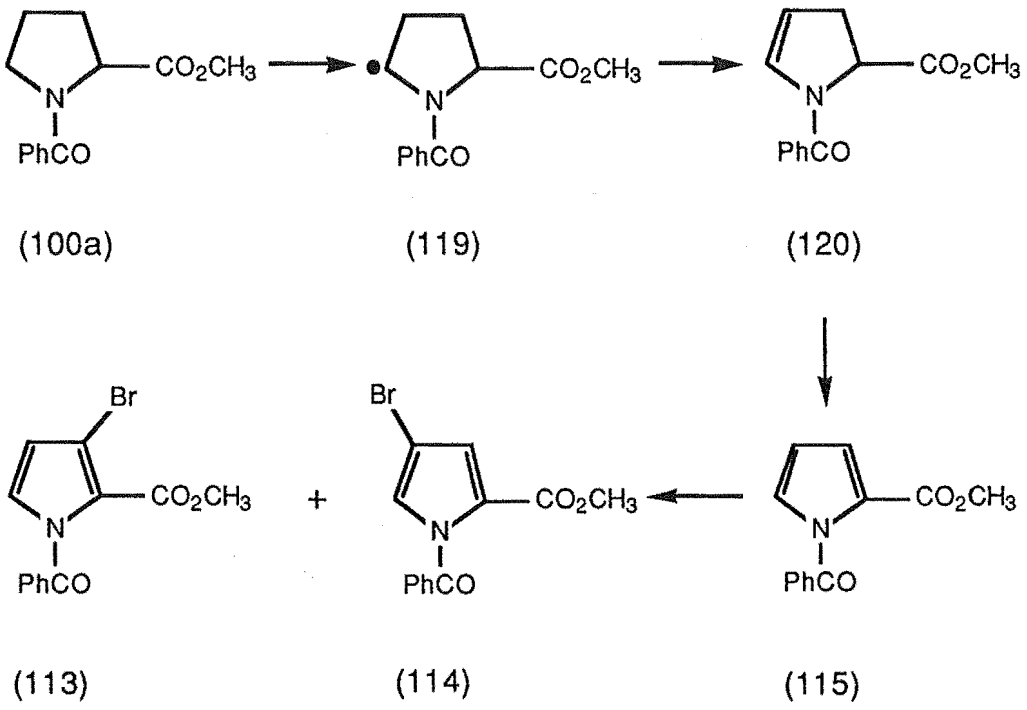
the dibromovaline derivative (76) with tri-*n* -butyltin hydride selectively afford the glycine derivative (93a) is also consistent with the rationale proposed above. Consideration of the structure of the radical (117) shows that non-bonding interactions associated with the 2-bromo-*iso* propyl group are greater than in the radical (34a). This interaction disrupts the planar configuration of the tertiary captodative radical (117) and destabilises the radical (117) relative to the secondary radical (116a). The secondary radical (116a) experiences minimum non-bonding interactions and hence retains it s planar configuration and captodative stabilisation.

The deuterium isotope effect observed for reaction of methyl pyro-glutamate (23a) with NBS shows that the reaction proceeds by hydrogen atom abstraction at the α-position. This is consistent with evidence that the reaction of methyl pyroglutamate (23a) with di-*t* -butyl peroxide involves reaction at the α-position.[34] That the rate of reaction of (23a) with NBS is faster than the corresponding rate of reaction for the glycine derivative (93a) implies that the radical (24) is more stable than the radical (116a). This is consistent with the rationale proposed above. The radical (24) readily assumes a planar conformation of the amido and carboxy substituents, required for captodative stabilisation. There are no nonbonding interactions between the α-alkyl substituent, indeed, the ring ensures that the amido group is held planar with respect to the radical centre. Formation of the radical (24) is also favoured by the release of gauche steric interactions between the methoxy-carbonyl group and the ring hydrogens in (23a). Thus the stabilisation of the radical (24) is enhanced relative to the glycyl radical (116a) and this is reflected in the relative rates of reaction of (23a) and (93a). Presumably radicals such as (24) have not been detected in studies of free radical reactions of proteins because of the low incidence of pyroglutamate residues in proteins, compared for example, to glycine residues.



(24)

The reaction of the proline derivative (100a) with NBS provided further evidence for the rationale proposed above. The deuterium isotope effects observed for the reaction of (100b) and (100c) with NBS show that reaction occurs predominantly at the δ-position with some reaction at the α-position. Further reaction proceeds, presumably, by a series of elimination reactions to give the pyrrole (115) which reacts by electrophilic aromatic substitution with bromine to give the bromo-substituted pyrroles (113) and (114) (Scheme 37). The proline derivative (100a) reacts with NBS 1.4 times faster than the glycine

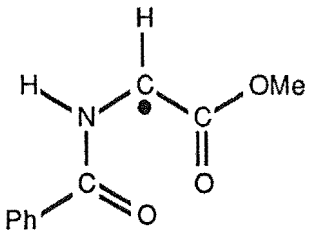


(100a) (119) (120)

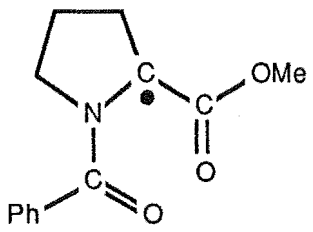
(113) (114) (115)

Scheme 37.

derivative (93a), but whereas the reaction of the glycine derivative (93a) gives the α-centred radical (116a) quantitatively, only a small proportion of the proline derivative (100a) reacts to form the α-centred radical (121). Hence the rate of formation of the glycyl radical (116a) is faster than that of the corresponding prolyl radical (121). The rationale proposed above indicates that the severe non-bonding interactions associated with planar conformations of (121), such as that shown in Figure 4, would disrupt the planar conformation required for captodative stabilisation and hence reduce the stability of the radical (121). The rate of formation of the δ-centred radical (119) from the proline derivative (100a) is similar to, or slightly faster than the rate of formation of the glycyl radical (116a) from the glycine derivative (93a). Stabilisation of the δ-centred radical (119) associated with relief of the gauche interactions in the reaction of the proline derivative (100a) with NBS more than compensates the loss of stabilisation of the methoxycarbonyl substituent. That the reaction of (100a) with NBS proceeds mainly through the δ-centred radical shows that steric interactions associated with (121) outweigh the stabilisation provided by the methoxycarbonyl substituent.



(116a)



(121)

Figure 4.

These results support the conclusion that the selective reaction of glycine residues in these and other free radical reactions of amino acid derivatives is

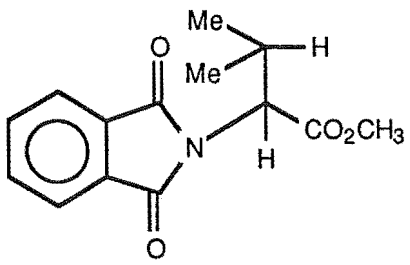
due to the stability of the radicals formed by atom transfer reactions. Non-bonding interactions with alkyl side chains may destabilise radicals formed by similar reactions of other amino acid derivatives. The nature of the alkyl substituent may alter the stability of the radical through bonding or non-bonding interactions with other parts of the molecule.

CHAPTER 4.

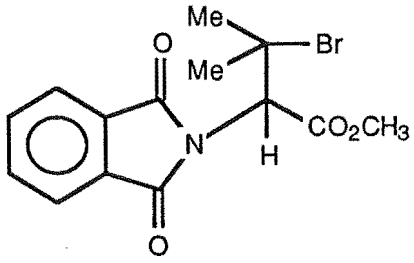
SYNTHESIS OF MODIFIED AMINO ACID DERIVATIVES.

Preliminary experiments were carried out to exploit results obtained in the work described in Chapters 1, 2 and 3 of this thesis.

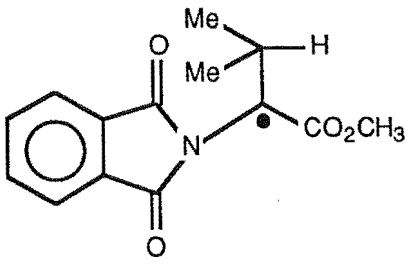
The work described in Chapters 1, and 2 indicates that the reaction of N-phthaloylvaline methyl ester (122) with NBS would be expected to result in reaction at the β-position to give the β-bromovaline derivative (123). The phthaloyl substituent is not a resonance-donating group hence the α-centred radical (124) would not be stabilised by the captodative effect of a resonance electron-donating substituent and a resonance electron-withdrawing substituent.



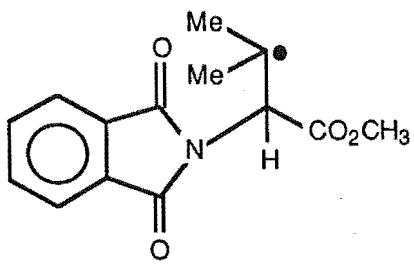
(122)



(123)



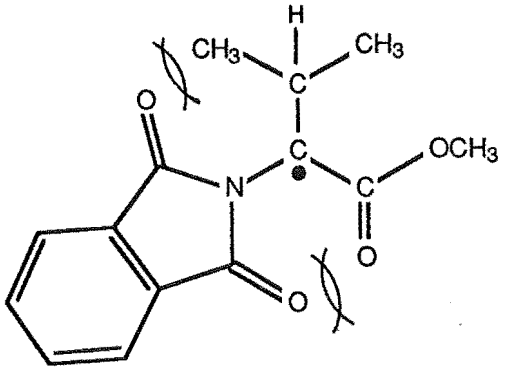
(124)



(125)

Additionally, steric interactions associated with planar conformations of the radical (124) such as that shown in Figure 5 would destabilise the radical (124) relative to the β-centred tertiary radical (125). Hence it was anticipated that

reaction of the valine derivative (122) with NBS would provide a method for selective functionalisation at the β-position. It was also thought that the greater selectivity of reactions with NBS would provide an advantage over functionalisation with sulphuryl chloride where γ-chlorination also occurs.



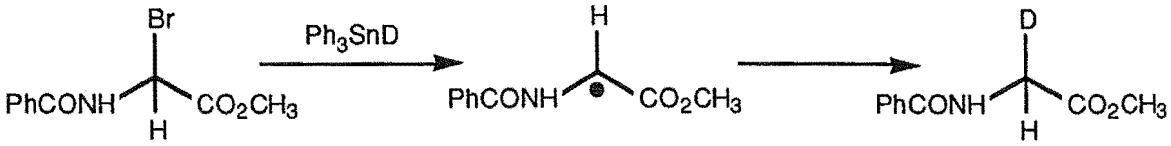
(124)

Figure 5.

A mixture of (R,S)-valine (49a) and N-phthaloyl acetate was suspended in a solution of sodium carbonate.[92] The suspension was acidified, filtered and the filtrate extracted with ethyl acetate. The organic extract gave an oil which was treated with methanol to which thionyl chloride had been added, and the solution left to stand overnight. Chromatography of the concentrated solution afforded N-phthaloylvaline methyl ester (122).[93] Photolysis of a mixture of N-phthaloyl-valine methyl ester (122) and NBS in refluxing carbon tetrachloride afforded the β-bromovaline derivative (123) in high yield. There was no evidence of any products derived from reactions at the α-position. Thus the reaction of the valine derivative (122) with NBS provides the basis for a method of selective β-functionalisation of valine derivatives.

The work presented in Chapter 3 of this thesis indicates that selective bromination of glycine residues in peptides could be expected for reaction of the peptides with NBS. This method has potential for use in the selective modification of glycine residues in peptides. The deuteration of the glycine

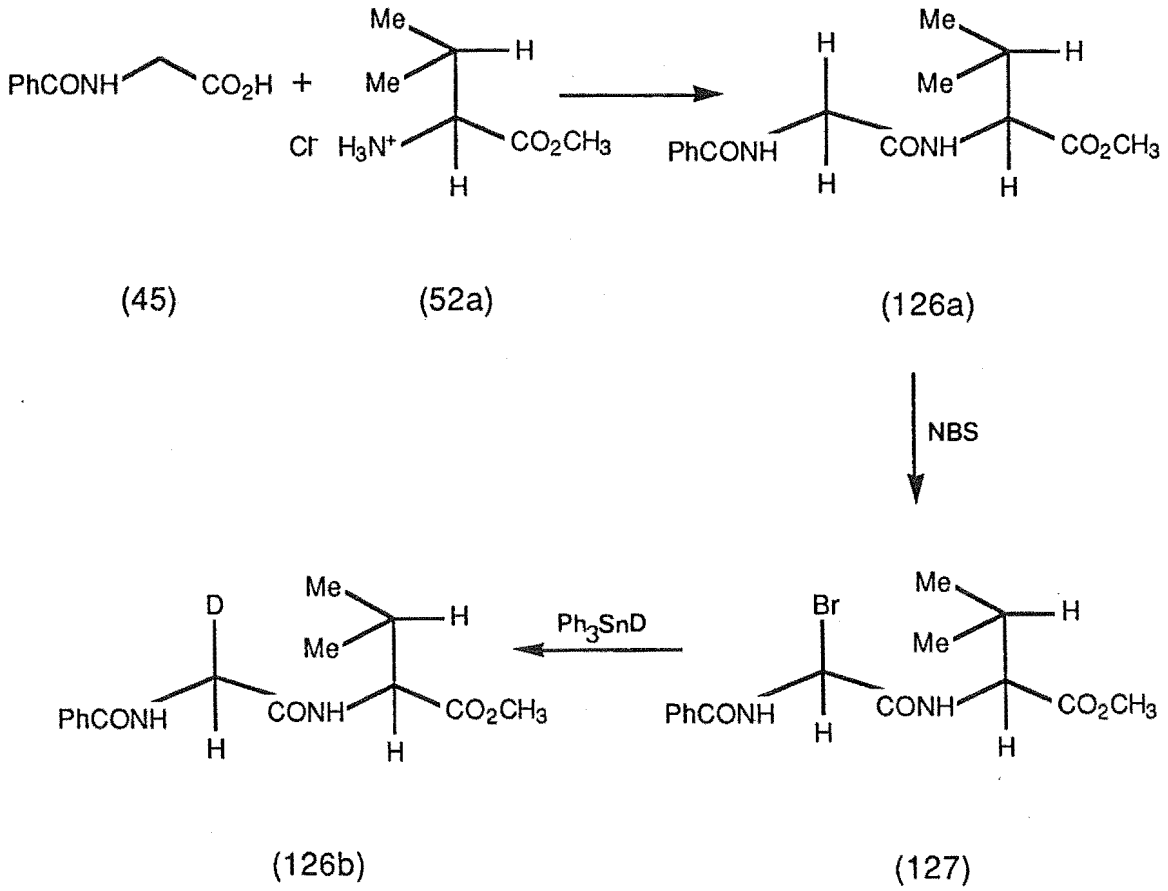
derivative (93a) was attempted by reaction of the α-bromoglycine derivative (112a) with triphenyltin deuteride. The α-bromoglycine derivative (112a), prepared as described in Chapter 3 from the glycine derivative (93a), was treated with triphenyltin deuteride[66,70] in benzene and left to stand at room temperature overnight. Chromatography afforded N-benzoyl-[2-^{2}H]-glycine methyl ester (93c) in 62% yield (Scheme 38). The ^{1}H n.m.r. spectrum showed a multiplet at δ 4.22-4.25 ppm which integrated for one proton and the mass spectrum gave a parent ion at *m/z* 194, consistent with the deuterated glycine derivative (93c). The deuterium content was established as 97%. Therefore it was expected that selective deuteration of peptides could be achieved through reaction with NBS followed by reaction with triphenyltin deuteride.



(112a) (116a) (93c)

Scheme 38.

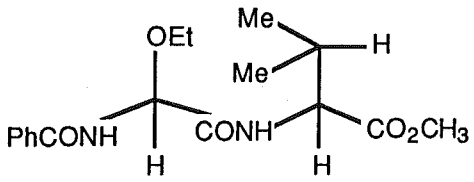
Valine methyl ester hydrochloride (52a), prepared as described in Chapter 1, and N-methylmorpholine were added to a cooled stirred solution of hippuric acid (45) in dichloromethane, to which N-methylmorpholine and dicyclohexylcarbodiimide had been added. Work up and recrystallisation from ethyl acetate / pet. ether afforded N-benzoylglycylvaline methyl ester (126a) (Scheme 39).[94]



Scheme 39.

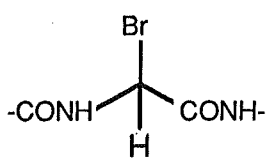
Photolysis of a mixture of the dipeptide (126a) and NBS, in refluxing carbon tetrachloride, afforded the α-bromoglycylvaline derivative (127). Formation of the α-bromoglycylvaline derivative (127) was determined by ^1H n.m.r. spectroscopy. The ^1H n.m.r. spectrum showed the presence of the benzamide, methyl ester groups and the valine residue. Absence of any signal at δ 4.22 ppm and the presence of a doublet at δ 6.95 ppm (d, J = 8 Hz), indicated that reaction had occurred to give the α-bromoglycylvaline derivative (127). Treatment of (127) with triphenyltin deuteride in benzene afforded, after chromatography, N-benzoyl-[2-^2H]-glycylvaline methyl ester (126b). The deuterated dipeptide was identified by ^1H n.m.r. spectroscopy and mass spectrometry. The ^1H n.m.r. spectrum showed the presence of the benzamide, methyl ester groups and the valine residue. The presence in the ^1H n.m.r.

spectrum of a doublet at δ 4.22 ppm was consistent with a monodeuterated glycine residue in the dipeptide. The ^1H n.m.r. spectrum showed no evidence of any deuterium being incorporated at the α-position of the valine residue. The mass spectrum gave a parent ion at *m/z* 293, consistent with the structure of (126b), and a deuterium content of 82%. Also isolated were the two diastereo-isomers of the α-ethoxygylcylvaline derivative (128). The stuctures of the diastereoisomers of the α-ethoxygylcylvaline derivative (128) were deduced by ^1H and ^{13}C n.m.r. spectroscopy and mass spectrometry. Both diastereoisomers gave spectra which indicate the presence of the benzamide, methyl ester groups and the valine residue. The presence in the ^1H n.m.r. spectrum of a doublet at δ 5.82 ppm is consistent with an electronegative substituent at the α-position of the glycine residue. The presence of a triplet at δ 1.30 ppm and a quartet at δ 3.82 ppm with integrals of three and two protons respectively, suggest an ethoxy substituent. This was confirmed by mass spectrometry.



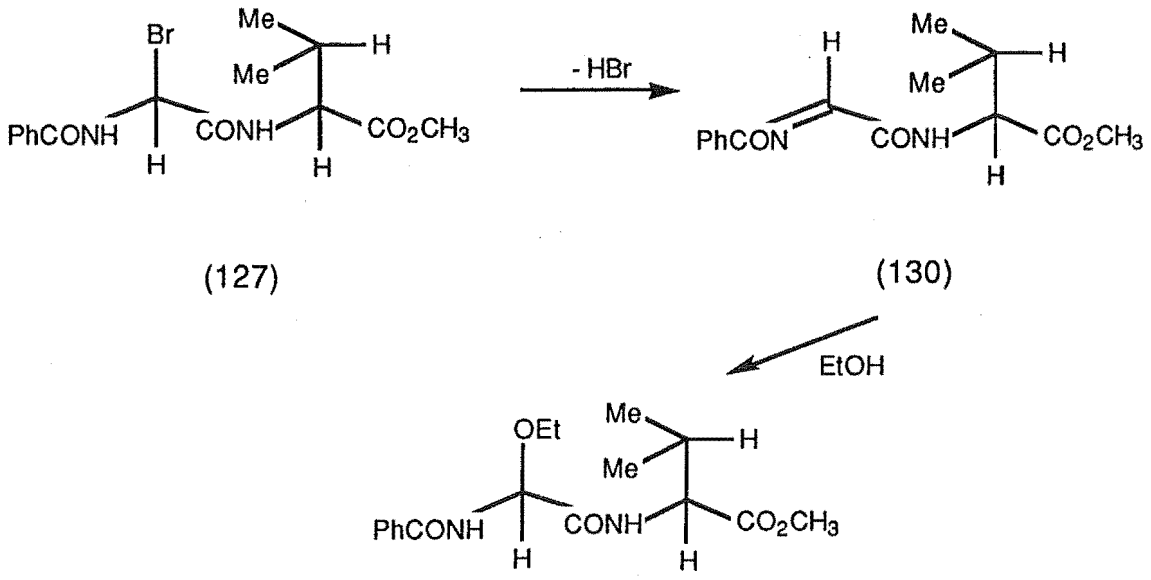
(128)

The reaction of the dipeptide (126a) with NBS and triphenyltin deuteride demonstrates that the selective functionalisation of dipeptides may be effected through formation of the α-bromoglycyl moiety (129) and it's subsequent reaction with a suitable reagent. The formation of the α-ethoxyglycylvaline derivative



(129)

(128) is thought to occur during chromatography by reaction with ethanol. Separation using ethyl acetate / dichloromethane mixtures on acidic silica could produce ethanol from the hydrolysis of ethyl acetate. Alternatively, ethanol is present as a stabiliser in chloroform which was used to apply the crude reaction mixture to the Chromatotron plate. Ethanol may add to the imine (130), produced by loss of HBr from any unreacted α-bromoglycylvaline derivative (127), to give the α-ethoxy derivative (128) (Scheme 40).



(127) (130)

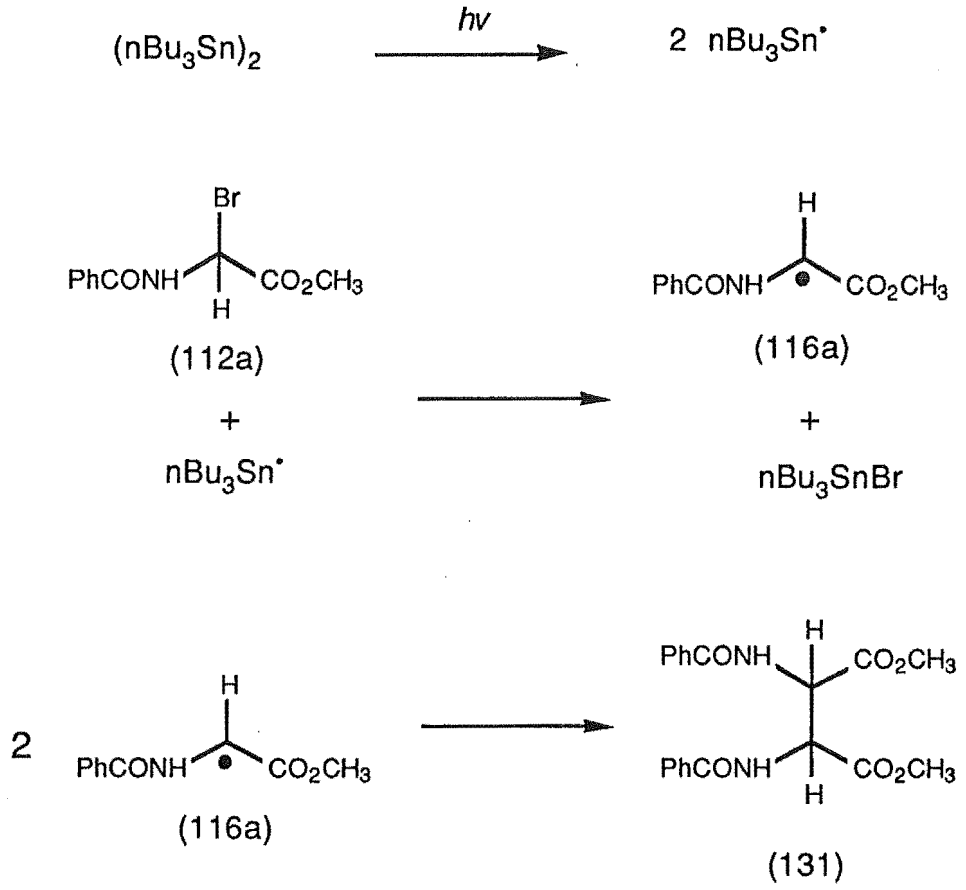
(128)

Scheme 40.

Having demonstrated that the selective bromination of glycine residues in peptides was possible, it was felt this step could be utilised in a method for cross-linking peptides. It was thought that the coupling of α-bromoglycine residues (129) could be used to cross-link peptides. Hence it was expected that the reaction of the α-bromoglycine derivative (112a) with hexabutylditin would give the dimer (131) (Scheme 41). Photolysis of hexabutylditin provides tri-*n* -butyltin

radicals. These radicals can react by bromine atom abstraction from the α-bromoglycine derivative (112a) to give tri-*n* -butyltin bromide and the α-centred radical (116a). Coupling of the radicals (116a) would give the dimer (131).

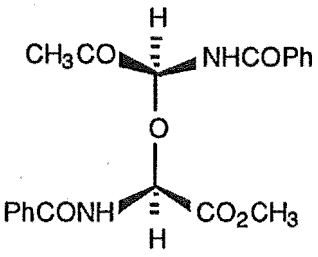
A mixture of the α-bromoglycine derivative (112a) and hexabutylditin was photolysed in refluxing benzene. The mixture was washed with aqueous sodium flouride solution to remove the tri-*n* -butyltin compounds generated in the reaction. The residue was examined by high field ^1H n.m.r. spectroscopy to determine the relative yields of the products. These products were isolated by chromatography.



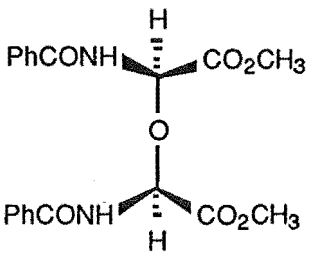
Scheme 41.

The expected products, the diastereoisomers of the dimer (131), were produced in low yield (5% each isomer). In addition to the expected products,

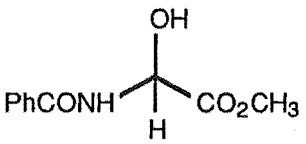
the two diastereoisomers of the ether (132) (30% each isomer), the α-hydroxyglycine derivative (133) (10%), and the α-benzamidoglycine derivative (134) (17%) were detected in the product mixture and isolated by chromatography. Also isolated, but not detected in the product mixture, was the α-ethoxyglycine derivative (135).



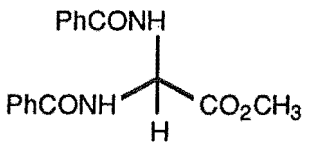
(S,S)-(132a)



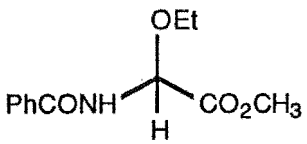
(R,S)-(132b)



(133)



(134)



(135)

The structures of the two diastereoisomers of the dimer (131) were deduced from their ^{1}H and ^{13}C n.m.r. spectra. The ^{1}H and ^{13}C n.m.r. spectra indicated the presence of the benzamide and methyl ester groups. The infra-red spectrum showed the amido and ester groups were intact. The presence of a doublet at δ 5.34 ppm in the ^{1}H n.m.r. spectrum which integrated for one proton indicated an α-substituent which was probably an alkyl substituent. The mass spectra gave parent ions of *m/z* 384 which suggested the compounds were the

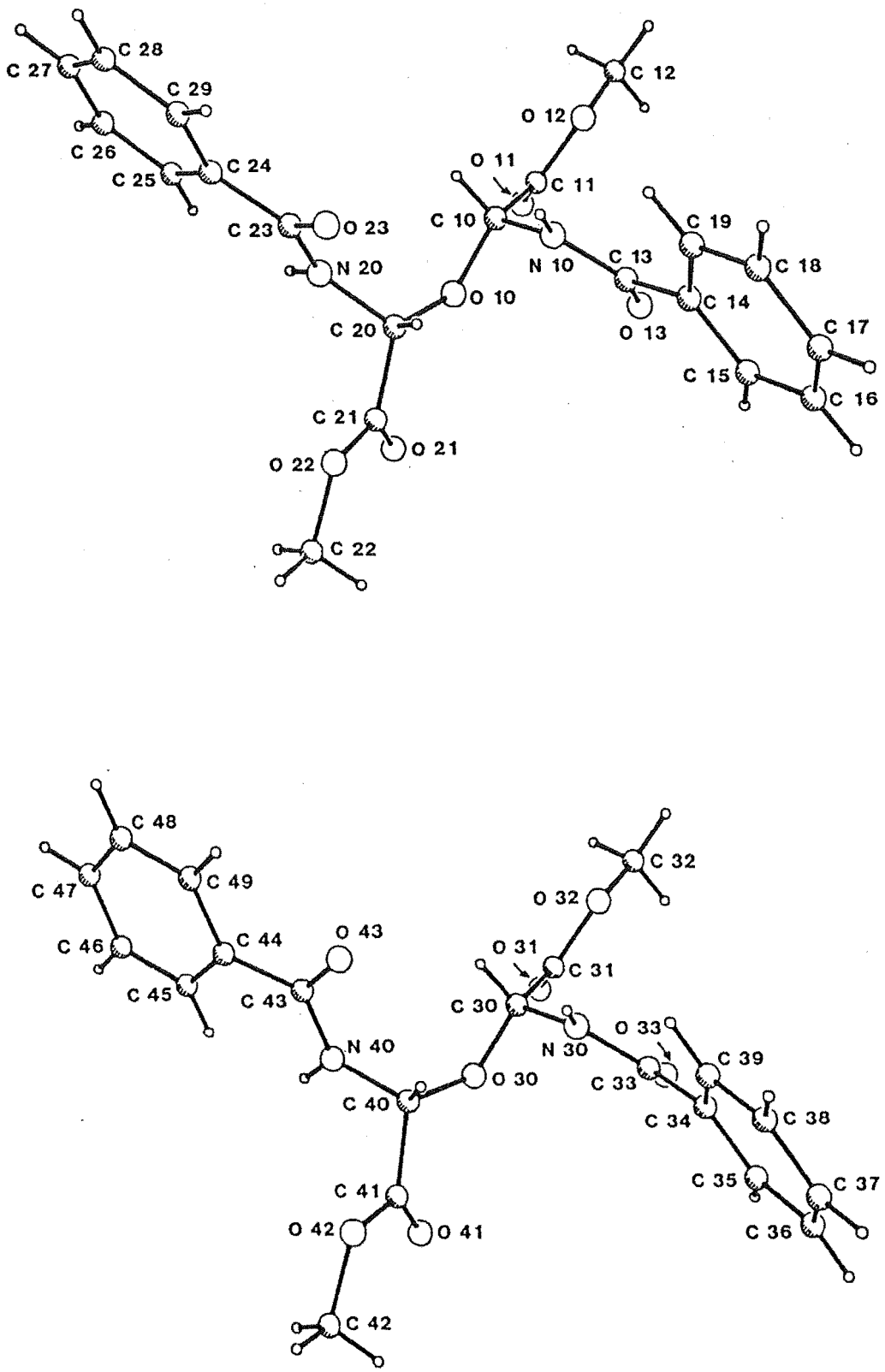


Figure 6. Perspective view and atom labelling of the two independent molecules.

dimers of the glycine derivative (131). This was confirmed by high resolution mass spectrometry and microanalysis. It was not possible to distinguish the diastereoisomers of (131) by X-ray crystallography because of a lack of suitable crystals.

The structure of the ether (132a) was determined by X-ray crystallography and shown to be that of the racemic diastereoisomer. The asymmetric unit contains two molecules of the ether and a disordered chloroform molecule. Figure 6 shows perspective views and atom labelling of the two independent molecules viewed in similar orientations (and both arbitrarily chosen as the R,R-enantiomer). Comparable bond lengths (Table 22) and bond angles (Table 23) (See Appendix) are similar within the two halves of each molecule and between the two independent molecules. These bond lengths and angles are similar to those found in structurally related compounds.[95]

However, significant torsional angle differences exist within the two halves of each molecule. For example the two hydrogens attached to C(10) and N(10) in molecule A are almost eclipsed (i.e. syn-coplanar) [H-N-C-H = 13.3(2)°] whilst the hydrogens attached to C(20) and N(20) are approximately anti-coplanar [H-N-C-H = 177.5(1)°]. Similarly conformational differences exist between the two molecules. For example the meanplanes through the two phenyl rings in molecule A are mutually inclined at an angle of 12.6(2)° whilst the corresponding value for molecule B is 30.5(2)°. These torsional differences are related to the molecular packing which is controlled by a complex network of hydrogen bonds. As listed in Table 24 all four independent amide hydrogens are hydrogen bonded to carbonyl oxygens of adjacent molecules. The microanalysis of (132a) is consistent with a formula of $(C_{20}H_{20}N_2O_7)_2.CHCl_3$ and the ^1H and ^{13}C n.m.r spectra are in accord with the structure.

The structure of the *meso* -diastereoisomer of the ether (132b) was deduced by comparison of it's ^1H and ^{13}C n.m.r. data with that of (132a) and confirmed by microanalysis. The ^1H and ^{13}C n.m.r spectra for the compound

(132b) indicated the presence of the benzamide and methyl ester groups with the chemical shift of the α-proton (δ 6.17, d, J = 8 Hz) showing a small displacement from the chemical shift value for the α-proton of the ether (132a) (δ 6.22, d, J = 8 Hz). Similarly the ^{13}C n.m.r. spectrum of (132b) was almost identical to that of (132a). The major difference is the displacement of the signal for the α-carbon of (132b) from δ 76.5 ppm to δ 75.4 ppm in (132a). The infra-red spectra shows the amido and ester groups are intact. The information derived from the spectra indicates that the compound (132b) is an α-substituted glycine derivative, and that the α-substituent is more electronegative than an alkyl group. Microanalysis of the compound (132b) is consistent with a formula of $C_{20}H_{20}N_2O_7$ which corresponds to the ether of the glycine derivative (132b).

The structures of the α-hydroxyglycine derivative (133) and the α-benzamidoglycine derivative (134) were deduced from their ^1H and ^{13}C n.m.r. spectra and mass spectra. It was deduced from the ^1H and ^{13}C n.m.r. spectra that the compound (133) is an α-substituted glycine derivative. The high field ^1H n.m.r. spectrum showed a doublet at δ 5.19 ppm, a triplet at δ 5.83 ppm and a doublet at δ 7.76 ppm. The doublet δ 5.19 ppm was assigned as the amide proton. Homonuclear decoupling experiments showed the proton represented by the triplet at δ 5.83 ppm is coupled to the amide proton and the proton represented by the doublet at δ 5.19 ppm. A D_2O exchange experiment resulted in the triplet at δ 5.83 ppm collapsing to a doublet and the loss of the doublet at δ 5.19 ppm. There was no exchange of the amide proton. These experiments showed that the proton represented by the signal at δ 5.19 ppm is labile and probably a hydroxyl proton. The triplet at δ 5.83 ppm represents the α-proton which is coupled to both the amide proton and the hydroxyl proton. The mass spectrum gave a peak at *m/z* 208 which corresponds to (M^+-H) for the formula $C_{10}H_{10}NO_4$ and microanalysis confirmed that the compound is the α-hydroxyglycine derivative (133).

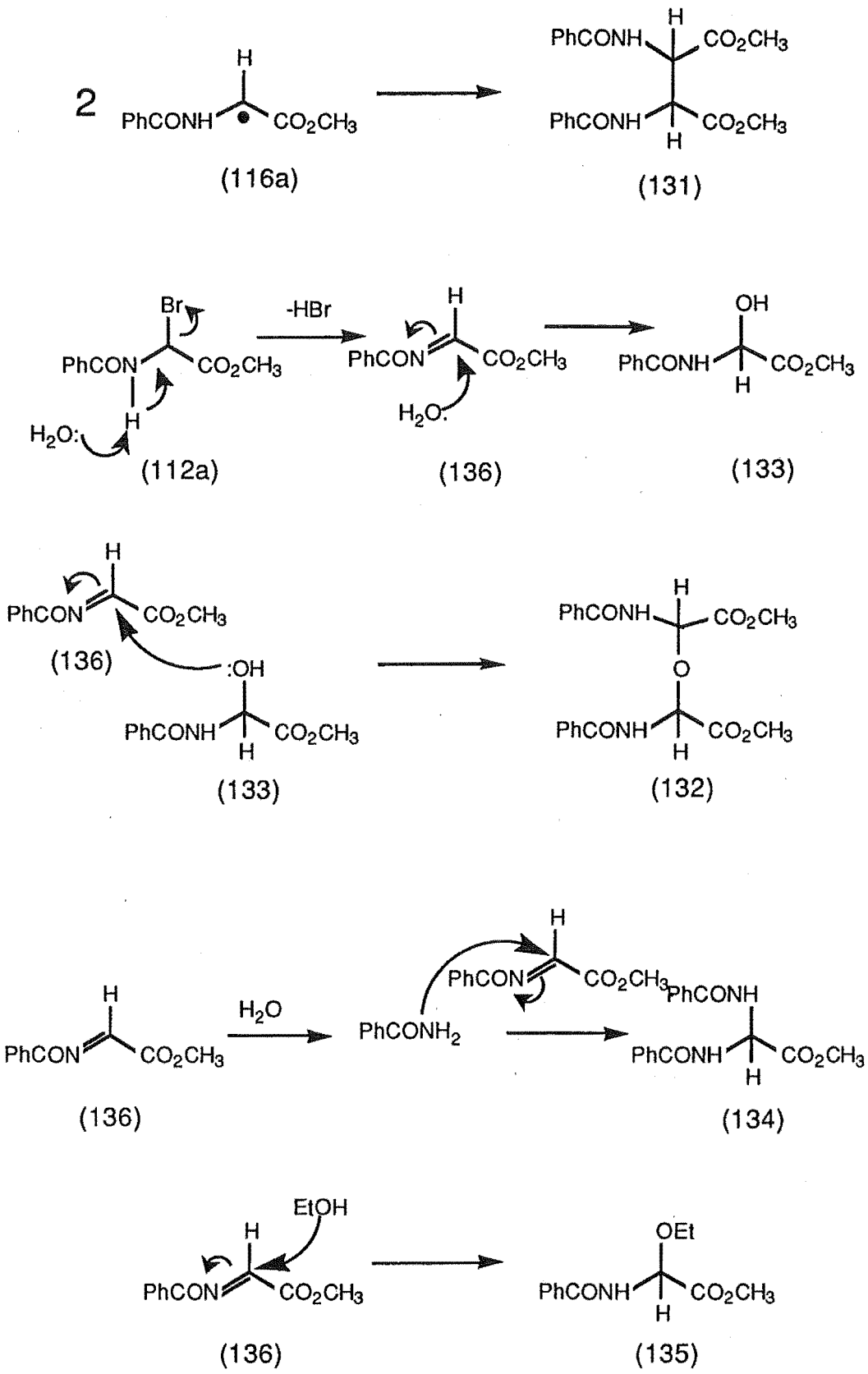
The α-benzamidoglycine derivative (134) gave ^1H n.m.r. signals for the

methoxycarbonyl protons, α-proton, amide proton and benzoyl protons, but the integral of the signals for the amide and benzoyl protons was twice the value expected for an α-substituted glycine derivative. A similar ratio of signal intensities was observed in the ^{13}C n.m.r. spectrum. The high resolution mass spectrum gave a peak at *m/z* 253 which corresponded to the αα-dibenzamido ion, $(PhCONH)_2CH^+$. This confirmed the α-substituent as a benzamido group and the structure was assigned as the α-benzamidoglycine derivative (134).

Also isolated, but thought to be an artefact of the separation process was the α-ethoxyglycine derivative (135). The structure of the α-ethoxyglycine derivative (135) was deduced from it's ^1H and ^{13}C n.m.r. spectra which showed the glycine derivative structure was intact with an electronegative substituent at the α-position. The presence of a triplet at δ 1.25 ppm and a quartet at δ 3.78 ppm, with integrals of three and two protons respectively, suggests an ethoxy substituent. This was confirmed by microanalysis.

The low yield of the dimers (131) prompted further attempts to improve the yield of these compounds. Repeated purging of the mixture of the α-bromoglycine derivative (112a) and hexabutylditin with nitrogen prior to photolysis gave an improved yield, as determined by ^1H n.m.r. spectroscopy, of the dimer (131) (15% each isomer). The improved yield was accompanied by a decrease in the yield of the oxydimers (132) (23% each dimer). Less α-benzamido derivative (134) (7%) was produced and there was an increase in the amount of α-hydroxyglycine derivative (133) (14%) produced. The experiment was repeated several times and although the product ratios varied slightly the above experiment provided the greatest yield of the dimers (131).

The reaction of the α-bromoglycine derivative (112a) with hexabutylditin was carried out in an effort to develop a method for coupling peptides at glycine residues. Formation of the diastereoisomers of the dimers (131) may be explained by the coupling of two α-centred glycine radicals (116a) (Scheme 42).


2
PhCONH
CO2CH3
(116a)
(131)
Br
-HBr
PhCON
H2O:
(112a)
(136)
OH
(133)
:OH
O
(132)
H2O
PhCONH2
(134)
EtOH
OEt
(135)

Scheme 42.

Formation of the other products may be attributed to the presence of water in the reaction mixture. The α-bromoglycine derivative (112a) readily loses HBr to form the imine (136). Addition of water to the imine (136) gives the α-hydroxyglycine derivative (133) which can add to another molecule of (136) to give the ether (131). Alternatively the imine (136) may hydrolyse to give benzamide which may add to another molecule of the imine (136) to give the α-benzamidoglycine derivative (134). Formation of the α-ethoxyglycine derivative (135) was thought to occur analogously to the formation of the α-ethoxy dipeptide (127) either, by reaction with ethanol produced from the hydrolysis of ethyl acetate during chromatography of the reaction mixture of the α-bromo-glycine derivative (113a) and hexabutylditin, or as a result of contact with ethanol present in chloroform as a stabiliser. Chloroform was used as a solvent to apply the crude reaction mixture to the chromatotron plate. Ethanol may add to the imine (136), produced by loss of HBr from any unreacted α-bromoglycine derivative (112a), to give the α-ethoxyglycine derivative (135).

When the reaction was carried out under strictly anhydrous conditions, the only products isolated were the isomers of the dimer (131) in *ca.* 70% yield. To achieve this condition all reaction vessels were flame dried, benzene and carbon tetrachloride were dried by standard methods. The α-bromoglycine derivative (112a) was prepared and handled under nitrogen at all times and hexabutylditin was dried by distillation. The rigourous exclusion of water ensured the coupling reaction between the α-centred radicals (116a) predominated.

These preliminary results indicate that functionalisation of glycine derivatives through formation of the α-bromoglycine moiety (129) is a useful synthetic route to modified peptides. Reaction of the α-bromo substituent with triphenyltin deuteride may be used to label peptides regioselectively. The coupling of peptides at glycine residues may be accomplished through reaction of α-glycine moieties (129) with hexabutylditin and the potential for reaction of (129) with suitable reagents to give modified peptides has yet to be exploited.

These preliminary results unveil a synthetic route to modified peptides worthy of further investigation.

CONCLUSION.

The work described in this thesis has examined atom transfer reactions of amino acid derivatives.

Reaction of the valine derivative (29a) with sulphuryl chloride has been shown to proceed by intermolecular regioselective hydrogen atom abstraction from the β-position. The possibility of intramolecular 1,5 hydrogen atom abstraction has been eliminated. This work shows that a similar regioselective hydrogen atom abstraction from the β-position of the valine derivative (5) to give the radical (12), postulated in penicillin biosynthesis, is chemically valid. The hydroxylation of valine residues (19) in peptides *via* formation of the radical (22) is also shown to be chemically valid.

The apparent anamoly between the reactions of the (23a) and (26) to give amidocarboxy-substituted radicals, and the contrasting regioselectivity displayed in the reaction of (29a) with sulphuryl chloride has been resolved. The work described in this thesis shows that amidocarboxy-substituted radicals such as (34a) are considerably more stable than the corresponding tertiary radical (32a), but that hydrogen atom transfer reactions may afford the less stable products if electrophilic radicals are involved in the hydrogen atom abstraction and if there is little development of radical character in the transition state of the reaction.

The preferential reactivity of glycine residues in free radical reactions of peptides and proteins has been examined through the study of a series of reactions of amino acid derivatives with NBS. Relative rates of reaction were determined and these are consistent with the previously reported but unexplained reactivity of glycine derivatives. This preferential reactivity may be attributed to the effect of steric interactions on planar conformations of amidocarboxy-substituted captodative radicals. This rationale is supported by

the measured rates of reaction of methyl pyroglutamate (23a) and the proline derivative (101a) with NBS.

The reactivity of the glycine derivative has been exploited synthetically. Selective functionalisation of glycine residues has been shown to be a potentially useful synthetic route to modified peptides. Regioselective deuterium labelling of the glycine residue in a dipeptide has been achieved as well as the coupling of a glycine derivative.

Extensions of this work could involve studies of the selective functionalisation of glycine residues in larger peptides. Also worthy of consideration is the possibility of asymmetric induction in reactions of glycine derivatives with NBS, leading to chiral glycine products.

EXPERIMENTAL.

All solvents used were technical grade. Petroleum ether (b.p. 50-70°C) was distilled before use. Diethyl ether (hereafter referred to as "ether") used in chromatography and synthesis was dried over anhydrous calcium chloride, dried over sodium wire and distilled. Hexane used for h.p.l.c. was washed with *c* HNO_3 and *c* H_2SO_4 for four days, washed with water, dried over anhydrous magnesium sulphate, percolated through alumina and distilled. Propan-2-ol used for h.p.l.c. was heated at reflux over magnesium and iodine and distilled. All other solvents were used without further purification. Commercially available reagents were used without further purification as were compounds previously prepared in the department, unless stated otherwise.

Silica gel, grade 923, was used for column chromatography. Small scale preparative chromatography was carried out on a Chromatotron (a centrifugally accelerated, radial thin layer chromatograph), model 7924, Harrison Research Inc. The Chromatotron rotors were coated with silica gel (PF 254 type 60, Merck: EM Laboratories Inc.) and were one or two millimetres in thickness. Compounds were eluted with ether / petroleum ether mixtures unless stated otherwise and non-coloured compounds were visualised under ultra-violet light. H.p.l.c. analyses were performed on a Shimadzu (LC-4A) HPLC with a Rheodyne (7125) injector, a Shimadzu ultra-violet spectrophotometric detector (SPD-2AS), and a Du Pont Zorbax cyanopropyl column (25 cm x 4.6 mm i.d.), using hexane / propan-2-ol mixtures as eluant. Product separations were achieved on a Du Pont Zorbax semi-preparative cyanopropyl column (25 cm x 9.4 mm i.d.), using hexane / propan-2-ol mixtures as eluant. The response factors of the compounds (29a-c), (30a-b), and (31a-c) for detection by the ultra-violet detector were considered sufficiently similar to allow for the direct comparison of the ratios of the compounds.

^1H n.m.r. spectra were recorded on either a Varian T60, Varian CFT-20 or

Varian XL-300 spectrometer, and were recorded for samples dissolved in carbon tetrachloride or deuterochloroform with tetramethylsilane as an internal reference or in D_2O with H_2O as an internal reference. ^{13}C n.m.r. spectra were obtained on a Varian CFT-20 n.m.r. spectrometer operating at 22 MHz or a Varian XL-300 n.m.r. spectrometer operating at 75 MHz. All spectra were recorded in deuterochloroform. Infrared spectra were recorded on a Shimadzu IR-27G spectrometer or a Pye Unicam SP3-300 spectrometer. Mass spectra were recorded on a AEI MS 902 spectrometer and a Kratos MS 9 spectrometer. Melting points were determined in an open-top capillary and are uncorrected. Microanalyses were performed by the microanalytical laboratory, University of Otago. Small scale distillations were performed in a Kugelrohr apparatus (Buchi GKR-50). Gas chromatography was performed on a Varian 3700 gas chromatograph with Chrompack XE-60-S-VAL-S-X-PEA or Carbowax 20M capillary columns and a flame ionization detector.

[3-^{2}H]-Valine (49b).

Dimethylpyruvic acid (47) was prepared according to the method of Ramage and Simonson.[61] Hippuric acid (45) (100 g, 0.56 mol), sodium acetate (50 g, 0.61 mol), acetic anhydride (250 ml, 3 mol) and acetone (250 ml) were heated at reflux for 7 h. The solution was washed with water (1500 ml), filtered and washed with aqueous sodium carbonate solution (10% w/v) to give crude 2-phenyl-4-*iso* propylidene oxazolone (46) as an orange solid (88 g, 78%). The oxazolone (46) was heated at reflux in *c* HCl (120 ml) for 6 h. Crystals of benzoic acid were removed by filtration and washed with *c* HCl (20 ml). The combined filtrate and wash was extracted with ether (4 x 20 ml) which was evaporated *in vacuo* to give a yellow oil. This oil was purified using a Kugelrohr apparatus to give dimethylpyruvic acid (47) (7.9 g, 15%), ^{1}H n.m.r. δ (CCl_4) 1.23 (d, J = 6 Hz, 6H), 3.30 (m, 1H), and 8.50 (br. s, 1H). Dimethylpyruvic acid (47) (7.9 g, 68 mmol) was dissolved in absolute ethanol to which sodium hydroxide (2.7 g, 68 mmol) had been dissolved. White plates of sodium dimethylpyruvate (48a) were filtered, washed with cold ethanol (3 x 5 ml) and air-dried (9.25 g, 98%), ^{1}H n.m.r. δ (D_2O) 1.23 (d, J = 6Hz, 6H), and 3.13 (m, 1H).

[3-^{2}H]-Valine (49b) was prepared from sodium dimethylpyruvate (48a) according to the method of Baldwin and Wan.[62] Sodium dimethylpyruvate (48a) (3.9 g, 28 mmol), dissolved in D_2O (15 ml, 0.75 mol), was added to D_2O (8 ml, 0.4 mol) in which sodium metal (0.30 g, 13 mmol) had been dissolved. The solution was left standing at room temperature for 2 h, the pH adjusted to 5.0 with dilute HBr and the solvent removed *in vacuo* at 25°C. The crude sodium α-keto-β-deuteroisovalerate (48b) was dissolved in anhydrous methanol (50 ml), to which ammonium bromide (5.70 g, 53 mmol) and sodium cyanoborohydride (3.16 g, 49 mmol) were added. After stirring under nitrogen for 4 days the cloudy suspension was carefully treated with *c* HCl (78 ml) and stirred for 30 minutes, the solvent removed *in vacuo* and freeze-dried. The white solid was suspended

in ethanol (150 ml), filtered and washed with cold ethanol (3 x 5 ml). The filtrate and washes were combined, treated with freshly distilled aniline (17 ml) and left to stand for 3 days. The crystals of [3-^{2}H]-valine (49b) were filtered and washed with cold ethanol (5 ml) and air-dried (1.32 g, 40%), ^{1}H n.m.r. δ (D_2O) 0.81 (s, 3H), 0.86 (s, 3H), and 3.44 (s, 1H).

(2S)-[3-^{2}H]-Valine (49b).

(2S)-[3-^{2}H]-Valine (49b) was prepared according to the method of Baldwin and Wan.[62] [3-^{2}H]-Valine (49b) (0.57 g, 4.8 mmol) was heated at reflux with acetic anhydride (1.9 ml, 17 mmol) in glacial acetic acid (5.0 ml) under nitrogen for 45 minutes. The mixture was cooled to 35°C and evaporated *in vacuo* to a syrup at 35°C. The crude N-acetyl-[3-^{2}H]-valine (50b) was dissolved in water (10 ml) and adjusted to pH 7.0 with dilute aqueous lithium hydroxide. Hog Acylase 1 (Sigma, Grade 2, 1580 units / mg, 32.3 mg) was added and the mixture incubated at 37°C for 18 h, then boiled briefly in the presence of a pinch of activated charcoal. On cooling, the suspension was filtered, washed with water (5 ml), the combined filtrate and wash adjusted to pH 6.0 with dilute HCl and freeze-dried. The solid was dissolved in water (4 ml), ethanol (24 ml) added, and the solution left to crystallise at 4°C. The crystals of (2S)-[3-^{2}H]-valine (49b) were filtered, washed with ethanol (1 ml) and ether (1 ml) and dried *in vacuo* (74 mg, 26%), ^{1}H n.m.r. δ (D_2O) 0.81 (s, 3H), 0.86 (s, 3H), and 3.44 (s, 1H). The chirality of (2S)-[3-^{2}H]-valine (49b) was assigned by g.l.c. analysis of the corresponding derivative (2S)-(29b) using a Chrompack XE-60-S-VAL-S-X-PEA capillary column as described below.

[2-^{2}H]-Valine (49c).

Valine (49a) (Aldrich, 2 g, 17 mmol) was heated at reflux with D_2O (8.0 ml, 0.4 mol) in acetic anhydride (148 ml, 1.32 mol) under nitrogen for 20 minutes.

More D_2O (18 ml, 0.9 mol) was carefully added, the solution cooled to room temperature and evaporated *in vacuo* to a pale yellow oil. This oil was heated at reflux in 6M HCl (40 ml) for 90 minutes, evaporated *in vacuo* to give a white solid which was suspended in ethanol (50 ml), filtered and washed with cold ethanol (3 x 5 ml). The filtrate and washes were combined, treated with freshly distilled aniline (7 ml) and left to stand for 3 days. The crystals of [2-^{2}H]-valine (49c) were filtered and washed with cold ethanol and air-dried (1.62 g, 80%), ^{1}H n.m.r. δ (D_2O) 0.83 (d, J = 6 Hz, 6H), and 2.3 (m, 1H).

(2S)-[2-^{2}H]-Valine (49c).

(2S)-[2-^{2}H]-Valine (49c) was prepared according to the methods of Baldwin[62] and Greenstein.[63] Valine (49a) (Aldrich, 1 g, 8.5 mmol) was heated at reflux with D_2O (4 ml, 0.2 mol) in acetic anhydride (74 ml, 0.66 mol) under nitrogen for 15 minutes. More D_2O (9.0 ml, 0.45 mol) was carefully added, the solution cooled and evaporated *in vacuo* to a syrup at 35°C. The crude N-acetyl-[2-^{2}H]-valine (50c) was dissolved in water (20 ml) and adjusted to pH 7.0 with dilute aqueous lithium hydroxide. Hog Acylase 1 (Sigma, Grade 2, 1580 units / mg, 65 mg) was added and the mixture incubated at 37°C for 18 h, then boiled briefly in the presence of a pinch of activated charcoal. On cooling, the suspension was filtered, washed with water (5 ml), the combined filtrate and wash adjusted to pH 6.0 with dilute HCl and freeze-dried. The solid was suspended in ethanol (50 ml), stirred for 20 minutes, filtered and washed with ethanol (5 ml). The filtrate and wash were combined, evaporated *in vacuo* and (2S)-[2-^{2}H]-valine (49c) recrystallised from aqueous ethanol (0.17 g, 34%), ^{1}H n.m.r. δ (D_2O) 0.83 (d, J = 6 Hz, 6H), and 2.34 (m, 1H). The chirality of (2S)-[2-^{2}H]-valine (49c) was assigned by g.l.c. analysis of the corresponding derivative (2S)-(29c) using a Chrompack XE-60-S-VAL-S-X-PEA capillary column as described below.

N-Benzoylvaline methyl ester (29a).

Valine (49a) (Sigma, 1.5 g, 2.8 mmol) was dissolved in anhydrous methanol (50 ml), to which thionyl chloride (12 g, 0.1 mol) had been added, and left to stand for 18 h. The solvent was removed *in vacuo* to give the crude valine methyl ester hydrochloride (52a) as a pale oil. This oil was dissolved in ethyl acetate (20 ml) and triethylamine (3.8 g, 38 mmol) added dropwise with stirring. Benzoyl chloride (1.9 g, 13.5 mmol) was added slowly and the suspension stirred for 5 h. Water (40 ml) was added and the solution stirred for 18 h. The organic layer was separated, the aqueous layer extracted with ethyl acetate (3 x 20 ml), the extracts combined, dried over anhydrous magnesium sulphate, filtered and the solvent removed *in vacuo*. The residue was purified by column chromatography on silica. Elution with ethyl acetate / dichloromethane (1:19) afforded N-benzoylvaline methyl ester (29a) which was recrystallised from ethyl acetate / hexane (1.49, 50%); m.p. 86-87°C (lit.,[96] 86°C); ^{1}H n.m.r. δ ($CDCl_3$) 0.98 (d, J = 6Hz, 6H), 2.25 (m, 1H), 3.74 (s, 3H), 4.75 (dd, J = 4 Hz, J = 8 Hz, 1H), 6.75 (br. d, J = 8 Hz, 1H), 7.25-7.55 (m, 3H), and 7.65-7.90 (m, 2H).

N-Benzoyl-(2R)-valine methyl ester (29a).

N-Benzoyl-(2R)-valine methyl ester (29a) was prepared from (2R)-valine (49a) (Aldrich) as described above for N-benzoylvaline methyl ester (29a); yield 51%; m.p. 108-109.5°C (lit.,[97] 110-111°C); ^{1}H n.m.r. δ ($CDCl_3$) 0.98 (d, J = 6 Hz, 6H), 2.20 (m, 1H), 3.75 (s, 3H), 4.77 (dd, J = 4 Hz, J = 8 Hz, 1H), 6.70 (br. d, J = 8 Hz, 1H), and 7.25-8.96 (m, 5H). Chiral purity was established as greater than 99% by g.l.c. comparison with racemic N-benzoylvaline methyl ester (29a) using a Chrompack XE-60-S-VAL-S-X-PEA capillary column. The ratio of the integrals of the signals for (2S)-(29a) : (2R)-(29a) from the g.l.c. trace was greater than 0.01 : 1.0.

N-Benzoyl-[3-^{2}H]-valine methyl ester (29b).

N-Benzoyl-[3-^{2}H]-valine methyl ester (29b) was prepared from [3-^{2}H]-valine (49b) as described above for N-benzoylvaline methyl ester (29a); yield 55%; m.p. 85-86°C (lit.,[96] m.p. 86°C); ^{1}H n.m.r. δ ($CDCl_3$) 1.00 (s, 6H), 3.80 (s, 3H), 4.78 (d, J = 8 Hz, 1H), 6.64 (br. d, J = 8 Hz, 1H), and 7.30-7.90 (m, 5H). Deuterium content was established by mass spectrometry as 87%.

N-Benzoyl-(2S)-[3-^{2}H]-valine methyl ester (29b).

N-Benzoyl-(2S)-[3-^{2}H]-valine methyl ester (29b) was prepared from (2S)-[3-^{2}H]-valine (49b) as described above for N-benzoylvaline methyl ester (29a); yield 48%; m.p. 108-109.5°C (lit.,[64] 110.5-111.0°C); ^{1}H n.m.r. δ ($CDCl_3$) 1.00 (d, J = 6 Hz, 6H), 3.80 (s, 3H), 4.80 (d, J = 8 Hz, 1H), 6.65 (br. d, J = 8 Hz, 1H), and 7.30-7.90 (m, 5H). Chiral purity was established as greater than 99% by g.l.c. comparison with racemic N-benzoylvaline methyl ester (29a) as described above. Deuterium content was established by mass spectrometry as 92%.

N-Benzoyl-[2-^{2}H]-valine methyl ester (29c).

N-Benzoyl-[2-^{2}H]-valine methyl ester (29c) was prepared from (2S)-[2-^{2}H]-valine (49c) as described above for N-benzoylvaline methyl ester (29a); yield 87%; m.p. 85-86°C (lit.,[96] 85-86°C); ^{1}H n.m.r. δ ($CDCl_3$) 0.98 (d, J = 6 Hz, 6H), 2.20 (m, 1H), 3.73 (s, 3H), 6.58 (br. s, 1H), and 7.25-7.90 (m, 5H). Deuterium content was established by mass spectrometry as 90%.

N-Benzoyl-(2S)-[2-^{2}H]-valine methyl ester (29c).

N-Benzoyl-(2S)-[2-^{2}H]-valine methyl ester (29c) was prepared from (2S)-[2-^{2}H]-valine (49c) as described above for N-benzoylvaline methyl ester (29a); yield 57%; m.p. 107-108°C (lit.,[64] 110.5-111.0°C); ^{1}H n.m.r. δ ($CDCl_3$) 1.00 (d, J = 6 Hz, 6H), 2.25 (m, 1H), 3.77 (s, 3H), 6.63 (br. s, 1H), and 7.25-7.90 (m, 5H).

Chiral purity was established as greater than 99% by g.l.c. comparison with racemic N-benzoylvaline methyl ester (29a) as described above. Deuterium content was established by mass spectrometry as 85%.

t-Butylhypochlorite.

t-Butylhypochlorite was prepared according to the method of Teeter and Bell.[67] Sodium hydroxide (20 g, 0.5 mol) was dissolved in water (150 ml) and cooled to 20°C. To this solution *t*-butanol (20.3 g, 0.27 mmol) in water (25 ml) was added. Chlorine gas was slowly bubbled through the solution for 45 minutes at 20°C. The upper oily layer was separated, washed with aqueous sodium carbonate (10% w/v) until the washings were no longer acidic, washed with an equal volume of water, dried over anhydrous magnesium sulphate and filtered (24 g, 80%). *t*-Butylhypochlorite was prepared immediately prior to use and was used without further purification or characterisation.

Potassium *t*-butoxide in *t*-butanol.

Potassium *t*-butoxide was prepared according to the method of Vogel.[98] Potassium metal (17 g, 2.3 mol) was carefully added to *t*-butanol (171 g, 2.3 mol). When the reaction had ceased the solution was stored under nitrogen.

N-Benzoyl-N-chlorovaline methyl ester (39a).

A solution of N-benzoylvaline methyl ester (29a) (1.0 g, 4.3 mmol) and *t*-butylhypochlorite (3 ml, 26 mmol) in toluene (20 ml) was left to stand for 16 h in the dark. Potassium *t*-butoxide in *t*-butanol (10 ml) was added to the stirred solution and 20 minutes the solvent was removed *in vacuo*. The residue was dissolved in chloroform (25 ml), washed with water (4 x 60 ml), dried over anhydrous magnesium sulphate and the solvent removed *in vacuo* to yield crude N-benzoyl-N-chlorovaline methyl ester (39a) as a red oil; ^{1}H n.m.r. δ

($CDCl_3$) 1.00 (d, J = 6 Hz, 3H), 1.03 (d, J = 6 Hz, 3H), 2.40 (m, 1H), 3.77 (s, 3H), 4.55 (d, J = 8 Hz, 1H), and 7.30-8.10 (m, 5H). All N-chloroamides were prepared immediately prior to use and were used without purification or characterisation.

N-Benzoyl-N-chloro-[3-^{2}H]-valine methyl ester (39b).

N-Benzoyl-N-chloro-[3-^{2}H]-valine methyl ester (39b) was prepared from N-benzoyl-[3-^{2}H]-valine methyl ester (29b) as described above for N-benzoyl-N-chlorovaline methyl ester (39a); ^{1}H n.m.r. δ ($CDCl_3$) 1.01 (s, 3H), 1.04 (s, 3H), 3.80 (s, 3H), 4.60 (s, 1H), and 7.30-8.10 (m, 5H).

N-Benzoyl-N-chloro-[2-^{2}H]-valine methyl ester (39c).

N-Benzoyl-N-chloro-[2-^{2}H]-valine methyl ester (39c) was prepared from N-benzoyl-[2-^{2}H]-valine methyl ester (29c) as described above for N-benzoyl-N-chlorovaline methyl ester (39a);^{1}H n.m.r. δ ($CDCl_3$) 1.00 (d, J = 6 Hz, 3H), 1.03 (d, J = 6 Hz, 3H), 2.40 (m, 1H), 3.75 (s, 3H), and 7.30-8.10 (m, 5H).

Photolysis of the N-chlorovaline derivatives (39a-c).

Irradiation of solutions of the crude N-chlorovaline derivatives (39a-c) (0.2 g, 0.7 mmol) in benzene (20 ml), in a Rayonet photochemical reactor equipped with 16 RPR 3000 lamps for 14 h at 25°C, gave mixtures of N-benzoylvaline methyl ester (29), N-benzoyl-3-chlorovaline methyl ester (30) and N-benzoyl-4-chlorovaline methyl ester (31). Analyses of the product mixtures and calculation of product ratios were carried out by h.p.l.c. and the products were purified by repeated semi-preparative h.p.l.c. The purified fractions were examined by ^{1}H n.m.r. spectroscopy.

The photolysis of (39a) afforded the following compounds: N-benzoyl-valine methyl ester (29a); m.p. 82-84°C (lit.,[96] 85-86°C), ^{1}H n.m.r. δ ($CDCl_3$) 0.98 (d, J = 6 Hz, 6H), 2.25 (m, 1H), 3.74 (s, 3H), 4.75 (dd, J = 4 Hz, J = 8 Hz, 1H), 6.75

(br. d, J = 8 Hz, 1H), and 7.25-7.90 (m, 5H), identical with that of an authentic sample;[36] N-benzoyl-3-chlorovaline methyl ester (30a) as an oil; ^{1}H n.m.r. δ ($CDCl_3$) 1.76 (s, 3H), 1.80 (s, 3H), 3.78 (s, 3H), 4.96 (d, J = 8 Hz, 1H), 6.95 (br. d, J = 8 Hz, 1H), and 7.20-7.90 (m, 5H); and the diastereoisomers of N-benzoyl-4-chlorovaline methyl ester (31a); ^{1}H n.m.r. δ ($CDCl_3$) (i) 1.09 (d, J = 7 Hz, 3H), 2.50 (m, 1H), 3.50 (m, 2H), 3.80 (s, 3H), 4.95 (dd, J = 4, 8 Hz, 1H)., 6.65 (br. d, J = 8 Hz, 1H), and 7.30-7.90 (m, 5H); and (ii) 1.14 (d, J = 7 Hz, 3H), 2.50 (m, 1H), 3.60 (m, 2H), 3.84 (s, 3H), 5.00 (dd, J = 5,9 Hz, 1H), 6.80 (br. d, J = 9 Hz, 1H), and 7.30-8.00 (m, 5H), identical with that of an authentic sample.[36]

(i) Product Study by H.p.l.c.

Solutions of each of the crude N-chlorovaline derivatives (39a-c) were irradiated, as described above, to determine deuterium isotope effects for the photolysis of the N-chlorovaline derivative (39). The ratios of the valine derivatives (29), (30), and (31) were determined by integration of the h.p.l.c. traces for the crude reaction mixtures of (39a-c) (Table 10). The ratios are the result of at least 6 replicate experiments. The yields of the γ-chlorovaline derivative (31) were assigned as unity in each case and the deuterium isotope effects for the photolysis of (39a-c) calculated from the relative ratios of the β-chlorovaline derivatives (Table 1).

Table 10: Relative Ratios of the Products of the Photolyses of (39a-c).

	Product ratios		
	(29)	(30)	(31)
(39a)	90.7 ± 3	6.9 ± 0.3	2.4 ± 0.1
(39b)	93.8 ± 3	3.8 ± 0.2	2.4 ± 0.1
(39c)	90.5 ± 3	7.1 ± 0.3	2.4 ± 0.1

(ii) Product Study by 1H N.m.r.

The deuterium isotope effects calculated above were confirmed by a ^1H n.m.r. study. A 1:1 mixture of N-benzoyl-N-chlorovaline methyl ester (39a) and N-benzoyl-N-chloro-[2-^2H]-valine methyl ester (39c) was prepared by treatment of a mixture of N-benzoylvaline methyl ester (29a) (300 mg, 1.28 mmol) and N-benzoyl-[2-^2H]-valine methyl ester (29c) (299 mg, 1.27 mmol) with *t*-butylhypochlorite (4 ml, 35 mmol) in toluene (10 ml). The solution was left to stand at room temperature in the dark for 16 h. Potassium *t*-butoxide in *t*-butanol (6 ml) was added to the stirred solution and after 20 minutes the solvent was removed *in vacuo*. The residue was dissolved in chloroform (20 ml), washed with water (4 x 80 ml), dried over anhydrous magnesium sulphate, filtered and the solvent removed *in vacuo*. The 1:1 mixture of N-benzoyl-N-chlorovaline methyl ester (39a) and N-benzoyl-N-chloro-[2-^2H]-valine methyl ester (39c) (450 mg, 1,7 mmol) in benzene (45 ml) was photolysed in a Rayonet Photochemical Reactor as described above. Column chromatography and semi-preparative h.p.l.c. afforded a mixture of 3-chlorovaline derivatives (30a) and (30b) (3 mg, 11 μmol) which was analysed by ^1H n.m.r. spectroscopy. A 1:1 mixture of N-benzoyl-N-chloro-[3-^2H]-valine methyl ester (39b) and N-benzoyl-N-chloro-[2-^2H]-valine methyl ester (39c), obtained as described above, was irradiated under identical conditions to the above photolyses. The mixture of 3-chlorovaline derivatives (30a) and (30b) were separated by column chromatography and semi-preparative h.p.l.c. and the purified fraction examined by ^1H n.m.r. spectroscopy. ^1H N.m.r. spectroscopy was used to monitor the ratio of the integrals of α-hydrogens to methyl ester hydrogens in the starting material and product. The integrals for the α-hydrogen were compared, with the integral of the methyl ester hydrogens as a reference (Table 11), to calculate the relative rates of production of the 3-chlorovaline derivative (30a) from N-benzoyl-N-chlorovaline methyl ester (39a) and N-benzoyl-N-chloro-[2-^2H]-valine methyl ester (39c). The reaction of

N-benzoyl-N-chloro-[2-^{2}H]-valine methyl ester (39c) to give N-benzoyl-[2-^{2}H]-3-chlorovaline methyl ester (30b) was used as an internal standard.

Table 11: Relative Rates of Production of 3-chlorovaline derivatives (30) from N-chlorovaline derivatives (39).

Compound	Integral Ratio α-proton / CO_2CH_3	Relative Rate of Production (30a) : (30b)
(29a) / (29c)	0.136 ± 0.005	1.04 ± 0.08
(30a) / (30b)	0.131 ± 0.005	
(29b) / (29c)	0.170 ± 0.006	0.55 ± 0.04
(30a) / (30b)	0.094 ± 0.004	

Triphenyltin hydride.[70]

To triphenyltin chloride (5 g, 13 mmol) in anhydrous ether (40 ml) lithium aluminium hydride (0.5 g, 0.13 mmol) was added. The slurry was stirred 16 h at room temperature, water (100 ml) was added and the solution stirred for 15 minutes. The solution was extracted into ether, dried over anhydrous magnesium sulphate and the solvent removed *in vacuo* to give a colourless liquid (4.0 g, 88%).

Reaction of N-benzoyl-N-chloro-(2S)-valine methyl ester (39a) and reduction of N-benzoyl-3-chlorovaline methyl ester (30a) with triphenyltin hydride.

A solution of N-benzoyl-(2S)-valine methyl ester (29a) (0.5 g, 2.1 mmol) was treated with *t*-butylhypochlorite (2 ml) as describd above to give N-benzoyl-N-chlorovaline methyl ester (39a) (0.4 g, 1.4 mmol, 66%). Photolysis of this sample of (39a) gave a mixture of N-benzoylvaline methyl ester (29a), N-

benzoyl-3-chlorovaline methyl ester (30a) and N-benzoyl-4-chlorovaline methyl ester (31a) as determined by h.p.l.c. A sample of the 3-chlorovaline derivative (30a) (10 mg, 37 μmol), isolated from the reaction mixture by column chromatography and semi-preparative h.p.l.c., was treated with triphenyltin hydride (0.5 ml, 1.4 mmol), in benzene (5 ml). The mixture was heated at reflux under nitrogen for 3 h. Analysis of the crude reaction mixture by g.l.c. using a Chrompack XE-60-S-VAL-S-X-PEA capillary column showed the product to be the pure enantiomer (2S)-(29a).

Reaction of N-benzoyl-(2R)-valine methyl ester (29a) and N-benzoyl-N-chloro-(2S)-valine methyl ester (39a).

A mixture of N-benzoyl-(2R)-valine methyl ester (29a) (0.75 g, 3.2 mmol) and N-benzoyl-N-chloro-(2S)-valine methyl ester (39a) (0.80 g, 3 mmol), prepared from (2S)-(29a) as described above, in benzene (20 ml) was heated at reflux with irradiation by a 250 W mercury lamp for 4 h. The 3-chlorovaline derivative (30a) (3.2 mg, 23 μmol), isolated by column chromatography and semi-preparative h.p.l.c. from the reaction mixture, was dissolved in benzene (3 ml) and heated at reflux with triphenyltin hydride (1 g, 3.2 mmol) for 2 h. A sample of the crude reaction mixture was analysed by g.l.c. using a Chrompack XE-60-S-VAL-S-X-PEA capillary column. The analysis showed the product to be a mixture of the (2R)- and (2S)-valine derivative (29a) in the ratio 1.28 : 1.0.

N-Benzoyl-*iso*-butylamine (54)

iso-Butylamine (53) (1.0 g, 14 mmol) in ethyl acetate (25 ml) was treated with triethylamine (4.1 g, 41 mmol) and benzoylchloride (2.3 g, 16 mmol). The mixture was stirred for 4 h, water (40 ml) added, and the mixture stirred for 16 h. The mixture was extracted with ethyl acetate (3 x 30 ml), the combined extracts dried over anhydrous magnesium sulphate and filtered. The solution was

concentrated *in vacuo* and chromatographed on silica. Elution with ethyl acetate / dichloromethane afforded N-benzoyl-*iso* -butylamine (54) which crystallised from ethyl acetate / pet. ether, (0.63 g, 26%), m.p. 46-48°C (lit.,[99] 55°C);^{1}H n.m.r. δ ($CDCl_3$) 1.08 (d, J = 5 Hz, 6H), 1.90 (m, 1H), 3.26 (dd, J = 5 Hz, J = 5 Hz, 2H), 6.3 (m, 1H), and 7.20-7.95 (m, 5H); *m/z* 177 (M^+, 26%), 162 (16), 134 (51), 122 (56), and 105 (100).

N-Benzoyl-*t* -butylamine (57a).

N-Benzoyl-*t*- butylamine (57a) was prepared as described above for N-benzoyl-*iso* -butylamine (54), yield 40%, m.p. 130 °C (lit.,[100] 134°C); ^{1}H n.m.r. δ ($CDCl_3$) 1.44 (s, 9H), and 7.20-7.95 (m, 6H); *m/z* 177 (M^+,42%), 162 (57), 134 (21), 122 (96), and 105 (100).

N-Benzoylaniline (57b).

N-Benzoylaniline (57b) was prepared as described above for N-benzoyl-*iso* -butylamine (54), yield 35%, m.p. 158-160°C (lit.,[101] 163°C), ^{1}H n.m.r. δ ($CDCl_3$) 7.0-8.1 (m, 11H); *m/z* 197 (M^+,11%), 167 (12), 139 (8), 115 (19), 106 (67), and 105 (100).

N-Benzoyl-N-chloro-*iso* -butylamine (55).

N-Benzoyl-*iso* -butylamine (54) (0.1 g, 0.56 mmol) in toluene (2 ml) was treated with *t* -butylhypochlorite (1 ml, 9 mmol) and the solution was left to stand at room temperature in the dark for 16 h. The stirred solution was treated with potassium *t*- butoxide in *t* -butanol (1 ml) and the solvent removed *in vacuo*. The residue was dissolved in chloroform (10 ml), washed with water (4 x 20 ml), dried over anhydrous magnesium sulphate and the solvent removed *in vacuo* to give the crude N-benzoyl-N-chloro-*iso*- butylamine (55) as a red oil, (57 mg, 48%), ^{1}H n.m.r. δ ($CDCl_3$) 0.90 (d, J = 6 Hz, 6H), 2.2 (m, 1H), 3.46 (d, J = 6 Hz, 2H), and

7.20-7.90 (m, 5H).

N-Benzoyl-N-chloro-*t*- butylamine (58a)

N-Benzoyl-N-chloro-*t* -butylamine (58a) was prepared as described above for N-benzoyl-N-chloro-*iso* -butylamine (55); ^{1}H n.m.r. δ ($CDCl_3$) 1.55 (s, 9H), and 7.20-7.55 (m, 5H).

N-Benzoyl-N-chloroaniline (58b)

N-benzoyl-N-chloroaniline (58b) was prepared as described above for N-benzoyl-N-chloro-*iso* -butylamine (55); ^{1}H n.m.r. δ ($CDCl_3$) 7.2-8.1 (m, 10H).

Photolysis of N-benzoyl-N-chloro-*iso* -butylamine (55) in the presence of N-benzoylvaline methyl ester (29a).

A mixture of N-benzoyl-N-chloro-*iso* -butylamine (55) (54 mg, 0.25 mmol) and N-benzoylvaline methyl ester (29a) (17 mg, 72 μmol) was irradiated in refluxing benzene (5 ml) as described above. The ^{1}H n.m.r. spectrum of the mixture was examined at intervals. There was no evidence of a signal for the α-proton of the N-chlorovaline derivative (39a), δ ($CDCl_3$) 4.55 (d, J = 8 Hz). The signal for the valine derivative (29a), δ ($CDCl_3$) 4.80 (dd, J = 4 Hz, J = 8 Hz) remained unchanged during the experiment.

Photolysis of N-benzoyl-N-chlorovaline methyl ester (39a) in the presence of N-benzoyl-*iso* -butylamine (54).

A mixture of N-benzoyl-N-chlorovaline methyl ester (39a) (52 mg, 0.2 mmol) and N-benzoyl-*iso* -butylamine (54) (20 mg, 0.1 mmol) was irradiated in refluxing benzene (5 ml) as described above. The ^{1}H n.m.r. spectrum of the mixture was examined at intervals. There was no evidence of a signal for the α-protons of the N-benzoyl-N-chloro-*iso* -butylamine (55), δ ($CDCl_3$) 3.46 (d, J = 6

Hz). The signal for the α-protons of the N-benzoyl-*iso* -butylamine (54), δ ($CDCl_3$) 3.26 (dd, J = 5 Hz, J = 5 Hz) remained unchanged during the experiment.

Photolysis of N-benzoyl-N-chloro-*t*- butylamine (58a) in the presence of N-benzoylvaline methyl ester (29a).

Irradiation of a mixture of N-benzoyl-N-chloro-*t*- butylamine (58a) (4.0 g, 18.9 mmol) and N-benzoylvaline methyl ester (29a) (0.5 g, 2.1 mmol) in benzene (50 ml) as described above afforded, after column chromatography, the 3-chlorovaline derivative (30a) (0.29 g, 51%) and a mixture of diastereoisomers of the 4-chlorovaline derivative (31a) (42 mg, 7%), identical with a sample obtained as described above.

Photolysis of N-benzoyl-N-chloroaniline (58b) in the presence of N-benzoyl-valine methyl ester (29a).

Irradiation of a mixture of N-benzoyl-N-chloroaniline (58b) (5.0 g, 22 mmol) and N-benzoylvaline methyl ester (29a) (0.5 g, 2.1 mmol) in benzene (50 ml) as described above afforded, after column chromatography, the 3-chlorovaline derivative (30a) (114 mg, 20%) and a mixture of diastereoisomers of the 4-chlorovaline derivative (31a) (13 mg, 2%), identical with a sample obtained as described above.

Reaction of N-benzoylvaline methyl ester (29a) and it's deuterated analogues (29b) and (29c) with sulphuryl chloride.

(i) Product Studies.

Product studies of the reactions of N-benzoylvaline methyl ester (29a), N-benzoyl-[3-^{2}H]-valine methyl ester (29b) and N-benzoyl-[2-^{2}H]-valine methyl ester (29c) were carried out for their reactions with sulphuryl chloride. Solutions

of each of the valine derivatives (29a-c) (2 mg, 8.5 μmol) in benzene (1 ml) were treated with sulphuryl chloride (5 mg, 63 μmol) and benzoyl peroxide (trace) and heated at reflux under nitrogen for 40 minutes. The solvent was removed under nitrogen, the residue dissolved and made up to 1 ml in benzene. The ratios of the 3-chlorovaline derivative (30) to the diastereoisomers of the 4-chlorovaline derivative (31) were determined, hence, using the production of the 4-chlorovaline derivatives (31) as an internal standard, the relative rates of formation of the 3-chlorovaline derivative (30) were determined. The results are presented in Table 1.

(ii) Rate of Consumption Studies.

Studies of the relative rates of reaction of the valine derivatives (29a-c) with sulphuryl chloride were carried out in competitive experiments. A mixture of N-benzoyl-(2R)-valine methyl ester (29a) (2 mg, 8.5 μmol) and N-benzoyl-(2S)-[3-^{2}H]-valine methyl ester (29b) (2 mg, 8.5 μmol) with *t*-butylbenzamide (57a) (5 mg, 28 μmol) as an internal standard, in benzene (2 ml), was treated with sulphuryl chloride (5 mg, 63 μmol) and benzoyl peroxide (trace). The mixture was heated at reflux, under nitrogen, for 40 minutes. The solvent was removed under nitrogen and the residue dissolved and made up to 1 ml in benzene. A mixture of N-benzoyl-(2R)-valine methyl ester (29a) (2 mg, 8.5 μmol) and N-benzoyl-(2S)-[2-^{2}H]-valine methyl ester (29c) (2 mg, 8.5 μmol) with *t*-butyl-benzamide (57a) (5 mg, 28 μmol) as an internal standard, in benzene (2 ml), was treated identically to the above mixture. The starting and product mixtures were analysed by g.l.c. using a Chrompack XE-60-S-VAL-S-X-PEA capillary column. The initial and final ratios of the amino acid derivatives (40a) and (93a) were determined by integration of the h.p.l.c. trace by a Hewlett Packard HP3390A integrator. The areas of the two substrates in the product mixtures were corrected using the internal standard by the factor $Area(STD_{SM}) / Area(STD_{Sample})$. The peak areas in the starting material and product mixtures were normalised by a factor of $100\% / Area_{SM}$. This converts the peak areas to

% unreacted substrate. The results were averaged and the error shown represents the spread of results. The error in the integration of the peaks is assumed to be negligible. The relative rates of reaction were calculated using equation 14 and are shown in Tables 12a and 12b.

Table 12a: Relative Rates of Reaction of (29a) and (29b) with sulphuryl chloride.

	Sample			
	1	2	3	4
$k_{(29b)} / k_{(29a)}$	0.77	0.85	0.78	0.76
	5	6	7	8
	0.84	0.84	0.82	0.76

$k_{(29b)} / k_{(29a)} = 0.80 \pm 0.04$

Table 12b: Relative Rates of Reaction of (29a) and (29c) with sulphuryl chloride.

	Sample			
	1	2	3	4
$k_{(29c)} / k_{(29a)}$	1.00	0.99	1.03	0.98

$k_{(29c)} / k_{(29a)} = 1.00 \pm 0.03$

Methyl 2-benzamido-3-methylbut-2-enoate (74)

Hippuric acid (45) (10 g, 56 mmol), sodium acetate (5 g, 61 mmol), acetic anhydride (25 ml, 0.22 mol) and acetone (30 ml, 0.5 mol) were heated at reflux for 7 h. The mixture was washed with water (150 ml), filtered and washed with aqueous sodium carbonate solution (10% w/v) to give the crude 2-phenyl-4-*iso*-propylidene oxazolone (46) as an orange solid (8.1 g, 72%). A mixture of the oxazolone (46) (2 g, 10 mmol) and aqueous potassium hydroxide solution (2M, 20 ml) was heated at 80°C for 1 h. The mixture was cooled, filtered and the solid

treated with *c* HCl and left to stand for 2 h. The solvent was removed *in vacuo* and the residue suspended in methanol (25 ml) to which sodium (0.3 g, 13 mmol) had been added. Chromatography afforded methyl 2-benzamido-3-methylbut-2-enoate (74)[61] (0.9 g, 39%); m.p. 130-133°C (lit., [102] 136-137°C), ^{1}H n.m.r. δ ($CDCl_3$); 1.90 (s, 3H), 2.20 (s, 3H), 3.76 (s,3H), and 7.15-7.95 (m, 6H), (lit.,[102] 1.88 (s, 3H), 2.18 (s, 3H), 3.74 (s, 3H), 7.33-7.65 (m, 3H), and 7.85-8.13 (m, 3H)).

N-Benzoyl-2,3-dichlorovaline methyl ester (75).

A mixture of methyl 2-benzamido-3-methylbut-2-enoate (74) (0.5 g, 2.1 mmol) and sulphuryl chloride (0.4 ml, 5.0 mmol) in carbon tetrachloride (50 ml), was left to stand at room temperature for 30 minutes, then concentrated to give N-benzoyl-2,3-dichlorovaline methyl ester (75) as a pale oil (0.53 g, 83%); ^{1}H n.m.r. δ ($CDCl_3$) 2.00 (s, 6H), 3.66 (s, 3H), and 7.3-8.0 (m, 6H); $\upsilon_{max.}$ 1 646 and 1 742 cm^{-1}; *m/z* 303 (M^+,1%), 270 (2), 269 (8), 268 (5), 267 (21), and 105 (100); *m/z* 267.0663 (M^+-HCl) [Calc. for $C_{13}H_{14}ClNO_3$ (M^+-HCl) *m/z* 267.0662].

Reaction of N-benzoyl-3-chlorovaline methyl ester (30a) with triphenyltin hydride.

A mixture of N-benzoyl-3-chlorovaline methyl ester (30a) (0.15 g, 0.56 mmol) and triphenyltin hydride (0.5 g, 1.4 mmol) in benzene (10 ml), was heated at reflux for 5 h, then cooled, concentrated, and chromatographed on silica. Elution with ethyl acetate / dichloromethane (1:9) afforded N-benzoylvaline methyl ester (29a) (89 mg, 68%). When a sample of (30a), obtained by treatment of (2S)-(29a) with sulphuryl chloride, was treated with triphenyltin hydride, the product was the pure enantiomer (2S)-(29a) as determined by g.l.c. analysis.

(2S)-[2-^{2}H]-Alanine (69b).

Alanine (69a) (BDH, 1.0 g, 11 mmol) was heated at reflux with D_2O (6 ml, 0.3 mol) in acetic anhydride (95 ml, 0.84 mol), under nitrogen, for 10 minutes. More D_2O (11 ml, 0.55 mol) was carefully added, the solution cooled and the solvent removed *in vacuo* at 35°C to give a yellow oil. The crude N-acetyl-[2-^{2}H]-alanine (70) was suspended in water (20 ml) and the solution adjusted to pH 7.0 with dilute aqueous lithium hydroxide. Hog Acylase I (Sigma, Grade 2, 1580 units / mg, 86 mg) was added and the mixture incubated at 37°C for 30 h. The mixture was boiled briefly in the presence of a pinch of activated charcoal, the cooled suspension filtered, washed with water (5 ml), the filtrate and wash combined, adjusted to pH 6.0 with dilute HCl and freeze-dried. The solid was suspended in ethanol (20 ml) and stirred for 20 minutes, filtered and washed with ethanol (5 ml). The filtrate and wash were combined, aniline (5 ml) added and the crystals filtered after 3 days. The crystals of (2S)-[2-^{2}H]-alanine (69b) were washed with cold ethanol (1 ml) and cold ether (5 ml) and air-dried (0.21 g, 42%). (2S)-[2-^{2}H]-alanine (69b) was derivatised without further purification or characterisation. The chirality of (2S)-(69b) was established by g.l.c. analysis of the derivative (2S)-(40b) as described below.

N-Benzoyl-(2S)-alanine methyl ester (40a).

(2S)-Alanine (69a) (BDH, 1.0g, 11 mmol) was dissolved in anhydrous methanol (50 ml) to which thionyl chloride (5.0 ml) had been added and the solution left to stand overnight. The solvent was removed *in vacuo* to give the crude alanine methyl ester hydrochloride (72) as a pale yellow oil. This was dissolved in ethyl acetate (50 ml) and triethylamine (5.0 ml, 36 mmol) added dropwise with stirring. Benzoyl chloride (1.3 ml, 11 mmol) was added slowly and the suspension stirred for 5 h. Water (50 ml) was added and the solution stirred for 16 h. The organic layer was separated, the aqueous layer extracted with

ethyl acetate (3 x 30 ml), the extracts combined, dried over anhydrous magnesium sulphate, filtered and the solvent removed *in vacuo.* The residue was purified by column chromatography on silica, eluting with ethyl acetate / pet. ether to give N-benzoyl-(2S)-alanine methyl ester (40a) (1.42 g, 61%), m.p. 55-57°C (lit.,[103] 56.5-57.5°C); ^{1}H n.m.r. δ ($CDCl_3$) 1.52 (d, J = 6 Hz, 3H), 3.80 (s, 3H), 4.78 (m, 1H), 6.85 (br. d, J = 8 Hz, 1H), and 7.25-7.95 (m, 5H), *m/z* 207 (M^+, 20%), 177 (5), 176 (9), and 148, (100). Chiral purity was established as greater than 99% by g.l.c. using a Chrompack XE-60-S-VAL-S-X-PEA capillary column. The ratio of the integrals of the signals for (2S)-(40a) : (2R)-(40a) from the g.l.c. trace was greater than 1.0 : 0.01.

N-Benzoyl-(2S)-[2-^{2}H]-alanine methyl ester (40b).

N-Benzoyl-(2S)-[2-^{2}H]-alanine methyl ester (40b) was prepared from (2S)-[2-^{2}H]-alanine (69b) as described above for N-benzoyl-(2S)-alanine methyl ester (40a), yield 57%; m.p. 55-57°C (lit.,[103] 56.5-57.5°C); ^{1}H n.m.r. δ ($CDCl_3$) 1.50 (s, 3H), 3.80 (s, 3H), 4.75 (m, residual H), 6.80 (br. s, 1H), and 7.20-7.90 (m, 5H); *m/z* 208 (M^+, 64%), 177 (25), 163 (4), and 148 (100). Deuterium content was established by mass spectrometry as 83%. Chiral purity was established as greater than 99% by g.l.c. using a Chrompack XE-60-S-VAL-S-X-PEA capillary column. The ratio of the integrals of the signals for (2S)-(40b) : (2R)-(40b) from the g.l.c. trace was greater than 1.0 : 0.01.

N-Benzoyl-(2R)-alanine methyl ester (40a).

N-Benzoyl-(2R)-alanine methyl ester (40a) was prepared from (2R)-alanine (69a) (Sigma) as described above for N-benzoyl-(2S)-alanine methyl ester (40a): yield 55%; ^{1}H n.m.r. δ ($CDCl_3$) 1.50 (d, J = 6 Hz, 3H), 3.75 (s, 3H), 4.76 (m, 1H), 6.76 (br. d, J = 8 Hz, 1H), and 7.20-7.90 (m, 5H); *m/z* 207 (M^+, 76%), 177 (29), 176 (41), 162 (4), and 148 (100). Chiral purity was established

as greater than 99% by g.l.c. comparison with a sample of N-benzoyl-(2S)-alanine methyl ester (40a) as described above.

N-Benzoyl-3-chloroalanine methyl ester (81).

N-Benzoylalanine methyl ester (40a) (0.1 g, 0.48 mmol) was treated with sulphuryl chloride (0.5 ml, 6.3 mmol) in refluxing benzene (5 ml), for 10 minutes. The solution was concentrated and chromatography on silica afforded N-benzoyl-3-chloroalanine methyl ester (81) (42 mg, 36%), m.p. 108-109°C (lit.,[75] 114.5-115°C), ^{1}H n.m.r. δ ($CDCl_3$) 3.80 (s, 3H), 4.00 (m, 2H), 5.20 (m, 1H), and 7.1-7.9 (m, 6H), (lit.,[75] 3.85 (s, 3H), 4.04 (dd, J = 6.0 Hz, J = 6.8 Hz, 2H), 5.20 (m, J = 6.0 Hz, J = 6.8 Hz, J = 15 Hz, 1H), 6.93-7.20 (br. d, J = 15 Hz, 1H), and 7.4-8.0 (m, 5H).

Alternatively, treatment of N-benzoylalanine methyl ester (40a) (0.5 g, 2.4 mmol) with *t*-butylhypochlorite (3 ml, 26 mmol) in toluene as described above gave N-benzoyl-N-chloroalanine methyl ester (73) (0.14 g, 24%). Photolysis of the N-chloroalanine derivative (73) as described above gave, after chromatography, the 3-chloroalanine derivative (81) (30 mg, 21%), identical to the sample described above.

N-Benzoyl-2,3-dibromovaline methyl ester (76).

A mixture of N-benzoylvaline methyl ester (29a) (0.5 g. 2.1 mmol) and NBS (1.2 g, 6.7 mmol) in carbon tetrachloride (50 ml) was heated at reflux under nitrogen, with irradiation from a 250 W mercury lamp, for 30 minutes. The cooled suspension was filtered and concentrated to give N-benzoyl-2,3-dibromovaline methyl ester (76) as an oil, (0.73 g, 88%); ^{1}H n.m.r. δ ($CDCl_3$) 2.18 (s, 6H), 3.67 (s, 3H), and 7.3-8.0 (m, 6H); $\upsilon_{max.}$ 1 686 and 1 745 cm^{-1}; *m/z* 395, 393, 391 (M^+,1,2 and 1% respectively), 314 (10), 313 (9), 312 (10), 311 (9), and 105 (100); *m/z* 392.9387 (M^+)[Calc. for $C_{13}H_{15}Br_2NO_3$ (M^+) *m/z* 392.9400].

Methyl 3-bromo-3-methyl-2-oxobutanoate (77).

A mixture of N-benzoylvaline methyl ester (29a) (0.5 g, 2.1 mmol) and NBS (0.8 g, 4.5 mmol) in carbon tetrachloride (20 ml), was heated at reflux under nitrogen, while irradiated with a 250 W mercury lamp, for 1 hour. The cooled suspension was filtered and and the solvent removed *in vacuo* to give an oil. The oil was chromatographed on silica, eluting with a gradient of ethyl acetate / pet. ether and gave methyl 3-bromo-3-methyl-2-oxobutanoate (77) (0.35 g, 79%), ^{1}H n.m.r. δ ($CDCl_3$) 1.98 (s, 6H), and 3.90 (s, 3H); $\upsilon_{max.}$ 1 720 and 1740 cm^{-1}; *m/z* 210 and 208 (M^+,10 and 11% respectively), 151 (49), 149 (56), 123 (100) and 121 (96); *m/z* 207.9726 (M^+) [Calc. for $C_6H_9BrO_3$ (M^+) 207.9735].

Hydrogenolysis of N-benzoyl-2,3-dibromovaline methyl ester (76).

A mixture of the dibromovaline derivative (76) (0.2 g, 0.51 mmol), sodium acetate (0.4 g, 4.9 mmol), acetic acid (0.4 g, 6.7 mmol), and 5% palladium on carbon (0.1 g), in methanol / water (4:1, 10 ml), was shaken under hydrogen (1 atm) for 2 h. Celite was added, and the solution was filtered and concentrated. The residue was dissolved in ethyl acetate, washed with water, dried over anhydrous magnesium sulphate and concentrated to give an oil, which was chromatographed on silica to give N-benzoylvaline methyl ester (29a) (16 mg, 13%) and methyl 2-benzamido-3-methylbut-2-enoate (74)[61,102] (47 mg, 40%), identical to a sample synthesised as described above.

Reaction of methyl 2-benzamido-3-methylbut-2-enoate (74) with NBS.

A mixture of methyl 2-benzamido-3-methylbut-2-enoate (74) (0.3 g, 1.3 mmol) and NBS (0.5 g, 2.8 mmol) in carbon tetrachloride (15 ml), was heated at reflux under nitrogen, while irradiated with a 250 W mercury lamp, for 30 minutes. The cooled suspension was filtered and concentrated to give N-benzoyl-2,3-dibromovaline methyl ester (76) (0.36 g, 70%), identical to a sample

obtained as described above.

Reaction of N-benzoylvaline methyl ester (29a) and it's deuterated analogues (29b) and (29c), with NBS.

A mixture of N-benzoyl-(2R)-valine methyl ester (29a) (10 mg, 0.043 mmol) and N-benzoyl-(2S)-[3-^{2}H]-valine methyl ester (29b) (10 mg, 0.043 mmol) with *t*-butylbenzamide (10 mg, 0.056 mmol) as an internal standard, in carbon tetrachloride (2 ml), was treated with NBS (10 mg, 0.056 mmol). The mixture was heated at reflux, under nitrogen, while irradiated with a 250 W mercury lamp, for 20 minutes. The suspension was cooled, the solvent removed under nitrogen and the residue made up to 1 ml in carbon tetrachloride. A mixture of N-benzoyl-(2R)-valine methyl ester (29a) (10 mg, 0.043 mmol) and N-benzoyl-(2S)-[2-^{2}H]-valine methyl ester (29c) (10 mg, 0.043 mmol), with *t*-butylbenzamide (10 mg, 0.056 mmol) as an internal standard, in carbon tetrachloride (2 ml), was treated identically to the above reaction. The starting and product mixtures were analysed by g.l.c. using a Chrompack XE-60-S-VAL-S-X-PEA capillary column. The initial and final ratios of the amino acid derivatives (40a) and (93a) were determined by integration of the h.p.l.c. traces by a Hewlett Packard HP3390A integrator. The areas of the two substrates in the product mixtures were corrected using the internal standard by the factor $Area(STD_{SM}) / Area(STD_{Sample})$. The peak areas in the starting material and product mixtures were normalised by a factor of $100\% / Area_{SM}$. This converts the peak areas to % unreacted substrate. The results were averaged and the error shown represents the spread of results. The error in the integration of the peaks is assumed to be negligible. The relative rates of reaction were calculated using equation (14) and are shown in Tables 13a and 13b.

Table 13a: Relative Rates of Reaction of (29a) and (29b) with NBS.

	Sample		
	1	2	3
$k_{(29b)} / k_{(29a)}$	0.98	0.99	1.02

$k_{(29b)} / k_{(29a)} = 1.00 \pm 0.03$

Table 13b: Relative Rates of Reaction of (29a) and (29c) with NBS.

	Sample			
	1	2	3	4
$k_{(29c)} / k_{(29a)}$	0.26	0.24	0.31	0.28

$k_{(29c)} / k_{(29a)} = 0.27 \pm 0.05$

Relative rates of reaction of N-benzoylvaline methyl ester (29a) and it's deuterated analogues (29b) and (29c) with di-*t*-butylperoxide.

A mixture of N-benzoyl-(2R)-valine methyl ester (29a) (2mg, 8.5 μmol) and N-benzoyl-(2S)-[3-^{2}H]-valine methyl ester (29b) (2 mg, 8.5 μmol), with *t*-butylbenzamide (2 mg, 11 μmol) as the internal standard, in benzene (2 ml), was treated with di-*t*-butylperoxide (Koch-Light, 1 ml, 4.8 mmol). The mixture was heated at reflux, while irradiated with 250 W mercury lamp, under nitrogen, for 5 days. A mixture of N-benzoyl-(2R)-valine methyl ester (29a) and N-benzoyl-(2S)-[2-^{2}H]-valine methyl ester (29c) was treated identically to the above reaction. The starting and product mixtures were analysed by g.l.c. and the initial and final ratios of the two valine derivatives (29a) and (29b) were determined by integration of the g.l.c. traces as described above. The relative rates of reaction were calculated using equation (14) and are shown in Tables 14a and 14b.

Table 14a: Relative Rates of Reaction of (29a) and (29b) with DtBP.

	Sample					
	1	2	3	4	5	6
$k_{(29b)} / k_{(29a)}$	0.45	0.52	0.51	0.56	0.49	0.63

$k_{(29b)} / k_{(29a)} = 0.53 \pm 0.1$

Table 14b: Relative Rates of Reaction of (29a) and (29c) with DtBP.

	Sample					
	1	2	3	4	5	6
$k_{(29c)} / k_{(29a)}$	0.71	0.70	0.63	0.64	0.64	0.69

$k_{(29c)} / k_{(29a)} = 0.67 \pm 0.04$

Relative rates of reaction of N-benzoylalanine methyl ester (40a) and it's deuterated analogue (40b) with sulphuryl chloride.

A mixture of N-benzoyl-(2R)-alanine methyl ester (40a) (2 mg, 9.7 μmol) and N-benzoyl-(2S)-[2-^{2}H]-alanine methyl ester (40b) (2 mg, 9.7 μmol), with *t*-butyl-benzamide (57) (2 mg, 11 μmol) as an internal standard, in benzene (2 ml), was treated with sulphuryl chloride (5 mg, 62 μmol) and benzoyl peroxide (trace). The mixture was heated at reflux under nitrogen for 45 minutes. The mixture was cooled, the solvent removed under nitrogen and the residue made up to 1 ml in benzene. The starting and product mixtures were analysed by g.l.c. and the initial and final ratios of the two alanine derivatives (40a) and (40b) were determined by integration of the g.l.c. traces as described above. The relative rates of reaction of the two alanine derivatives were calculated using equation (14) and are shown in Tables 15.

Table 15: Relative Rates of Reaction of (40a) and (40b) with sulphuryl chloride.

	Sample					
	1	2	3	4	5	6
$k_{(40b)} / k_{(40a)}$	0.82	0.88	0.83	0.83	0.89	0.91

$k_{(40b)} / k_{(40a)} = 0.86 \pm 0.05$

Relative rates of reaction of N-benzoylalanine methyl ester (40a) and it's deuterated analogue (40b) with N-bromosuccinimide.

A mixture of N-benzoyl-(2R)-alanine methyl ester (40a) (2 mg, 9.7 μmol) and N-benzoyl-(2S)-[2-^{2}H]-alanine methyl ester (40b) (2 mg, 9.7 μmol), with *t*-butylbenzamide (57) (2 mg, 11 μmol) as an internal standard, in carbon tetrachloride (2 ml), was treated with NBS (3 mg, 17 μmol). The mixture was heated at reflux, with irradiation by a 250 W UV lamp, under nitrogen, for 40 minutes. The mixture was cooled, the solvent removed under nitrogen and the residue made up to 1 ml in carbon tetrachloride. The starting and product mixtures were analysed by g.l.c. and the initial and final ratios of the two alanine derivatives (40a) and (40b) were determined by integration of the g.l.c. traces as described above. The relative rates of reaction of the two alanine derivatives were calculated using equation (14) and are shown in Tables 16.

Table 16: Relative Rates of Reaction of (40a) and (40b) with NBS.

	Sample			
	1	2	3	4
$k_{(40b)} / k_{(40a)}$	0.50	0.57	0.50	0.54

$k_{(40b)} / k_{(40a)} = 0.53 \pm 0.04$

Reaction of N-benzoyl-2,3-dichlorovaline methyl ester (75) with triphenyltin hydride.

A solution of N-benzoyl-2,3-dichlorovaline methyl ester (75) (100 mg, 0.33 mmol) and triphenyltin hydride (90 mg, 0.31 mmol) in benzene (3 ml) was left to stand at room temperature under nitrogen for 30 minutes, then concentrated and chromatographed in silica. Elution with a gradient of ethyl acetate / pet. ether gave N-benzoyl-3-chlorovaline methyl ester (30a) as an oil[36] (65 mg, 73%). Analysis of crude reaction mixtures by h.p.l.c. showed that the ratio of (30a) to (29a) produced in this reaction was greater than 100:1.

Attempted synthesis of methyl 2-(benzoylimino)-isovalerate (78).

Valine (49a) (2 g, 17 mmol) was dissolved in methanol (80 ml) to which thionyl chloride (10 ml, 0.13 mol) had been added and the solution left to stand at room temperature for 16 h. The solvent was removed *in vacuo* and the residue washed with methanol (25 ml) several times and evaporated *in vacuo* . The crude valine methyl ester hydrochloride (52) (2.1 g, 12.5 mmol) was dissolved in water (20 ml) and treated with sodium bicarbonate (1.2 g, 14.3 mmol). The solution was extracted into chloroform (3 x 50 ml), the extracts combined and the solvent removed *in vacuo* . The residue was dissolved in ether (25 ml) and *t* -butylhypochlorite (4 ml, 35 mmol) added to the cooled, stirred solution which was left to stand at room temperature for 15 minutes. The solvent was removed, the residue dissolved in chloroform (20 ml), washed with dilute HCl (0.1 M, 20 ml), washed with saturated sodium chloride solution and the solvent removed *in vacuo* . To a cooled, stirred solution of the residue in ether (60 ml) 1,8-diazabicyclo[5.4.0]undec-7-ene (2 ml, 13 mmol) was added and the mixture stirred for 10 minutes. The mixture was filtered and the cooled, stirred filtrate treated with triethylamine (1.8 g, 12.8 mmol) followed by benzoyl chloride (1.9 g, 16 mmol) dropwise. The mixture was stirred at room temperature for 2 h, filtered

and the filtrate evaporated *in vacuo* . The residue was dissolved in chloroform, washed with water (5 ml), dried over anhydrous magnesium sulphate and the solvent removed *in vacuo* to give an oil. There was no evidence in the ^{1}H n.m.r. spectrum of formation of the imine (78) and attempts to isolate the imine (78) from the mixture using a Kugelrohr apparatus were unsuccessful.

[2,2-$^2H_2$]-Glycine (89b).

Glycine (89a) (BDH, 0.6 g, 8 mmol) was heated at reflux with D_2O (6 ml, 0.3 mol) in acetic anhydride (57 ml, 0.56 mol), under nitrogen, for 10 minutes. More D_2O (6.5 ml,0.33 mol) was carefully added, the solution cooled, and the solvent removed *in vacuo* . The residue was heated at reflux in HCl (6M, 30 ml) for 90 minutes, evaporated *in vacuo* to give a white solid which was suspended in ethanol (30 ml), filtered and washed with cold ethanol (2 x 5 ml). The filtrate and washes were combined, treated with freshly distilled aniline (3 ml) and left to stand for 24 h. The crystals of [2,2-$^2H_2$]-glycine (89b) were filtered, washed with cold ethanol and air-dried (0.52 g, 84%). The deuterated amino acid (89b) was derivatised without further characterisation or purification.

N-Benzoylglycine methyl ester (93a).

Hippuric acid (45) (BDH, 5.0 g, 28 mmol) was dissolved in anhydrous methanol (100 ml) to which thionyl chloride (16 ml, 0.22 mol) had been added and the solution was left to stand at room temperature for 16 h. The solvent was evaporated *in vacuo* to give a white solid. Purification by column chromatography on silica with ethyl acetate / dichloromethane (1:9) as eluant gave N-benzoylglycine methyl ester (93a), (2.64 g, 49%); m.p. 78-79°C (lit.,[104] 82-83°C); ^1H n.m.r. δ ($CDCl_3$) 3.80 (s, 3H), 4.26 (d, J = 4 Hz, 2H), (br. d, J = 4 Hz, 1H), and 7.25-7.95 (m, 5H); *m/z* 193 (M^+, 20%), 162 (4), 161 (3), 149 (1), 148 (1), 135 (49), 117 (5), 106 (59), and 105 (100).

N-Benzoyl-[2,2-$^2H_2$]-glycine methyl ester (93b).

[2,2-$^2H_2$]-Glycine (89b) (0.52 g, 6.8 mmol) was dissolved in anhydrous methanol (20 ml) to which thionyl chloride (1.6 ml, 22 mmol) had been added and the solution left to stand overnight. The solvent was removed *in vacuo* to give the

crude [2,2-$^2H_2$]-glycine hydrochloride (93) as a white solid. This was suspended in ethyl acetate (20 ml) and triethylamine (1.9 ml, 13.6 mmol) added dropwise with stirring. Benzoyl chloride (0.8 ml, 6.8 mmol) was added slowly and the suspension stirred for 4 h. Water (20 ml) was added and the solution stirred for 16 h. The organic layer was separated, the aqueous layer extracted with ethyl acetate (3 x 20 ml), the extracts combined, dried over anhydrous magnesium sulphate, filtered and the solvent removed *in vacuo* . The residue was purified by column chromatography on silica with ethyl acetate / dichloromethane (1:19) as eluant. Recrystallisation from ethyl acetate / hexane afforded N-benzoyl-[2,2-$^2H_2$]-glycine methyl ester (93b) (0.32 g, 24%), m.p. 77-79°C (lit.,[104] 82-83°C); ^1H n.m.r. δ ($CDCl_3$) 3.83 (s, 3H), 4.26 (d, J = 4 Hz, residual H), 6.70 (br. s, 1H), and 7.25-7.95 (m, 5H); *m/z* 195 (M^+, 64%), 194 (11), 164 (16), 163 (5), 137 (20), 136 (100), 135 (41), and 134 (23). Deuterium content was established at 83% dideuterated and 14% monodeuterated by mass spectrometry.

N-Benzoyl-2-bromoglycine methyl ester (112a).

A mixture of N-benzoylglycine methyl ester (93a) (0.5 g, 2.6 mmol) and N-bromosuccinimide (0.5 g, 2.8 mmol), in carbon tetrachloride (40 ml), was heated at reflux, while irradiated with a 250 W mercury lamp, under nitrogen, for 60 minutes. The suspension was cooled in a salt / ice bath for 30 minutes, filtered and the solvent removed *in vacuo* to give the crude N-benzoyl-2-bromoglycine methyl ester (112a) which was used without further purification, ^1H n.m.r. δ ($CDCl_3$) 3.86 (s, 3H), 6.56 (d, J = 8 Hz, 1H), 7.10-7.95 (m, 6H), (lit.,[89] δ 3.90 (s, 3H), 6.71 (d, J = 10.5 Hz, 1H), 7.50 (m, 4H), and 7.85 (m, 2H); ^{13}C n.m.r. δ (CCl_4) 49.3, 52.9, 127.3, 128.3, 132.0, 132.4, 164.0, and 166.9.

Reaction of N-benzoyl-2-bromoglycine methyl ester (112a) with nBu_3SnH.

A sample of N-benzoyl-2-bromoglycine methyl ester (112a), prepared

from N-benzoylglycine methyl ester (93a) (0.1 g, 0.52 mmol) as described above, was shown to be free of any unreacted N-benzoylglycine methyl ester (93a) by comparison with a sample of the starting material by h.p.l.c. and ^{1}H n.m.r. spectroscopy. The sample of (112a) was dissolved in benzene (10 ml), treated with tri-*n*- butyltin hydride (0.3 g, 0.86 mmol) and left to stand at room temperature for 16 h. Analysis by h.p.l.c. showed the presence of a compound with an identical retention time as (93a) and ^{1}H n.m.r. spectroscopy showed the presence of a doublet (J = 4 Hz) at δ 4.25 ppm, consistent with the formation of N-benzoylglycine methyl ester (93a).

Reaction of N-benzoyl-2-bromoglycine methyl ester (112a) and N-benzoyl-2,3-dibromovaline methyl ester (76) with nBu_3SnH.

A mixture of N-benzoyl-2-bromoglycine methyl ester (112a), prepared from N-benzoylglycine methyl ester (93a) (0.1 g, 0.52 mmol) as described above, and N-benzoyl-2,3-dibromovaline methyl ester (76) (0.2 g, 0.51 mmol) was treated with tri-*n* -butyltin hydride (0.16 g, 0.46 mmol) in benzene and let stand at room temperature for 5 h. Analysis of the starting material by h.p.l.c. and $_1$H n.m.r. spectroscopy showed no evidence of any N-benzoylglycine methyl ester (93a). Analysis of the reaction mixture by h.p.l.c. showed the presence of a compound with an identical retention time as (93a). ^{1}H N.m.r. spectroscopy showed the presence of a doublet (J = 4 Hz) at δ 4.25 ppm, consistent with the formation of N-benzoylglycine methyl ester (93a). There was no evidence of a signal at δ 4.78 (d, J = 9Hz) consistent with the formation of the β-bromovaline derivative (118).

Methyl pyroglutamate (23a)

A chilled solution of pyroglutamic acid (98a) (2.5 g, 19 mmol) in ether / methanol (1:1, 50 ml) was carefully treated with a solution of diazomethane[105] in

ether until the solution remained pale yellow in colour. The solution was left to stand for 16 h, the remaining solvent removed *in vacuo* and the residue purified using a Kugelrohr apparatus to give methyl pyroglutamate (23a) as an oil, (2.5 g, 90%); b.p. 130-140°C, 0.5 mm Hg, Kugelrohr (lit., [106] 101-103°C, 0.15 mm); ^{1}H n.m.r. δ ($CDCl_3$) 2.05-2.60 (m, 4H), 3.75 (s, 3H), 4.05-4.40 (m, 1H), and 7.12 (m, 1H); *m/z* 143 (M^+, 49%), 115 (3) 85 (28), and 84 (100).

Methyl [2-^{2}H]-pyroglutamate (23b).

Glutamic acid (94a) (2 g, 13.4 mmol) was heated at reflux with D_2O (7 ml, 0.35 mol) in acetic anhydride (100 ml, 1.06 mol), under nitrogen, for 15 minutes. More D_2O (14 ml, 0.7 mol) was carefully added, the solution cooled, and the solvent removed *in vacuo* . The residue was heated at reflux in HCl (6M, 80 ml) for 90 minutes and the solvent removed *in vacuo* . The residue was dissolved in anhydrous methanol (50 ml) in which dry HCl gas (0.5 g, 13.7 mmol) had been dissolved. The solution was left to stand at room temperature for 30 minutes and the solvent removed *in vacuo* . The residue was dissolved in anhydrous methanol (50 ml) and ammonium hydroxide solution (1M, 13.6 ml) was added to the solution which was left to stand for 10 minutes. The solvent was removed *in vacuo* and crystallisation from aqueous ethanol afforded γ-ethyl glutamic acid (97a), (0.83 g, 35%), m.p. 184-185°C (lit.,[84] 185°C), ^{1}H n.m.r. δ ($CDCl_3$) 1.20 (t, J = 6 Hz, 3H), 1.95-2.70 (m, 4H), and 4.1 (q, J = 6 Hz, 2H); ^{13}C n.m.r. δ ($CDCl_3$) 26.4, 30.6, 53.2, 54.8, 174.5, and 176.0. γ-Ethyl glutamic acid (97a) (0.22 g, 1.2 mmol) was dissolved in methanol (40 ml) saturated with dry ammonia and left to stand at room temperature for 9 h. The solvent was removed *in vacuo* , ethanol (40 ml) added, evaporated again, and the residue dissolved in dilute HCl (1M, 4 ml). Acetone (50 ml) was added, the suspension filtered and the filtrate evaporated *in vacuo* . The residue was dissolved in anhydrous methanol and diazomethane in ether added until the solution remained a pale yellow colour. The solution was left to stand at room temperature for 16 h and the solvent

removed *in vacuo* . The residue was purified using a Kugelrohr apparatus to give methyl [2-^{2}H]-pyroglutamate (23b) as an oil, (0.11 g, 61%); ^{1}H n.m.r. δ ($CDCl_3$) 2.15-2.60 (m, 4H), 3.68 (s, 3H), 4.20-4.30 (m, residual H), and 6.68 (s, 1H); *m/z* 144 (M^+, 65%), 143 (43), 116 (30), 115 (18), 99 (16), 98 (7), 89 (12), 88 (18), 87 (20), and 86 (100). Deuterium content was established at 62% by mass spectrometry.

Reaction of methyl pyroglutamate (23a) with NBS.

A mixture of methyl pyroglutamate (23a) (0.1 g, 0.7 mmol) and N-bromosuccinimide (0.15 g, 0.8mmol) in carbon tetrachloride (25 ml), was heated at reflux while irradiated with a 250 W mercury lamp under nitrogen for 1 hour. The suspension was chilled in an ice / salt bath for 1 hour, filtered and the solvent removed *in vacuo* . All attempts to resolve the product mixture by chromatography were unsuccessful and analysis of the product mixture by ^{1}H n.m.r. spectroscopy and h.p.l.c. failed to identify any products.

[2-^{2}H]-Proline (99b).

Proline (99a) (BDH, 1.0 g, 8.7 mmol) was heated at reflux with D_2O (4 ml, 0.2 mol) in acetic anhydride (60 ml, 0.63 mol), under nitrogen, for 10 minutes. More D_2O (8.5 ml, 0.43 mol) was added carefully, the solution cooled and the solvent evaporated *in vacuo* at 35°C. The residue was heated at reflux in 6M HCl (40 ml) for 90 minutes, the solvent evaporated *in vacuo* and [2-^{2}H]-proline (99b) recrystallised from hot ethanol, (0.79 g, 78%), m.p. 191-193°C (lit.,[63] 205°C). The deuterated amino acid (99b) was derivatised without further purification or characterisation. The deuterium content was established for the derivative (100b) as described below.

[2,5,5-$^2H_3$]-Proline (99c).

[2,5,5-$^2H_3$]-Proline (99c) was synthesised according to the methods of

Leitch,[85] Keefer and Fodor,[86] and Lijinsky *et. al.* [87] (2S)-Proline (99a) (BDH, 2.0 g, 17.4 mmol) was dissolved in *c* HCl (2.0 ml) and water (10 ml). Sodium nitrite (2.0 g, 29 mmol) in water (10 ml) was added slowly to the cooled, stirred proline solution which was left to stand for 90 minutes, the solvent removed *in vacuo* and the N-nitrosoproline (101) extracted with acetone. N-Nitrosoproline (101) was recrystallised from chloroform (0.92 g, 39%), m.p. 98-100°C (lit.,[87] 99-100°C). N-Nitrosoproline (101) (0.36 g, 2.7 mmol) was treated with D_2O (5 ml, 0.25 mol) in which sodium (0.25 g, 11 mmol) had been dissolved, and heated at reflux, under nitrogen, for 4 h. *c* HCl (5 ml) was added and the solution heated at reflux under nitrogen for 8 h. Evaporation of the solvent *in vacuo* yielded the crude [2,5,5-$^2H_3$]-proline hydrochloride (102) which was derivatised without further purification or characterisation. The deuterium content was established for the derivative (100c) as described below.

N-Benzoyl-(2S)-proline methyl ester (100a).

(2S)-Proline (99a) (BDH, 2.0 g, 17.4 mmol) was dissolved in anhydrous methanol (67 ml) to which thionyl chloride (10 ml, 0.13 mol) had been added and the solution left to stand at room temperature for 16 h. The solvent was removed *in vacuo* to give a pale yellow oil. This oil was dissolved in ethyl acetate (50 ml) and triethylamine (7.2 ml, 51 mmol) added dropwise with stirring. Benzoyl chloride (2.4 ml, 20 mmol) was added slowly and the suspension stirred for 5 h. Water (40 ml) was added and the solution stirred for 16 h. The organic layer was separated, the aqueous layer extracted with ethyl acetate (3 x 20 ml), the extracts combined, dried over anhydrous magnesium sulphate, filtered and the solvent removed *in vacuo*. The solid was recrystallised from benzene / pet. ether to give N-benzoyl-(2S)-proline methyl ester (100a), (2.0 g, 49%); m.p. 88-89°C (lit.,[107] 89-89.5°C); ^1H n.m.r. δ ($CDCl_3$) 1.88-2.34 (m, 4H), 3.51-3.68 (m, 2H), 3.78 (s, 3H), 4.68 (dd, J = 6, J = 8 Hz, 1H), 7.37-7.44 (m, 3H), and 7.56-7.59 (m, 2H); ^{13}C

n.m.r. δ ($CDCl_3$) 25.1, 29.5, 49.9, 52.3, 59.2, 127.3, 128.3, 128.4, 130.2, 169.7, and 172.8; *m/z* 233 (M^+, 8%), 184 (20), 117 (25), and (105 (100).

N-Benzoyl-[2-^{2}H]-proline methyl ester (100b).

N-Benzoyl-[2-^{2}H]-proline methyl ester (100b) was prepared from [2-^{2}H]-proline (99b) as described above for N-benzoyl-(2S)-proline methyl ester (100a); yield 38%; m.p. 55-57°C; ^{1}H n.m.r. δ ($CDCl_3$) 1.80-2.34 (m, 4H), 3.50-3.70 (m, 2H), 3.78 (s, 3H), 4.68 (dd, J = 6, J = 8 Hz, residual H), 7.28-7.44 (m, 3H), and 7.56-7.59 (m, 2H); ^{13}C n.m.r. δ ($CDCl_3$) 25.3, 29.2, 49.8, 52.2, 59.1, 126.3, 128.2, 128.3, 130.1, 169.6, and 172.8; $\upsilon_{max.}$ 1 740, 1 620 cm^{-1}; *m/z* 234 (M^+,16%), 217 (3), 203 (2), 186 (6), 185 (56), 184 (5), 119 (4), 118 (25), 117 (5), and 105 (100); *m/z* 234.1110 (M^+) [Calc. for $C_{13}H_{14}NO_3D$ (M^+) 234.1115]. Deuterium content was established by mass spectrometry as 83%.

N-Benzoyl-[2,5,5-$^2H_3$]-proline methyl ester (100c).

N-Benzoyl-[2,5,5-$^2H_3$]-proline methyl ester (100c) was prepared from crude [2,5,5-$^2H_3$]-proline hydrochloride (102) as described above for N-benzoyl-(2S)-proline methyl ester (100a); yield 44%; m.p. 61-63°C; ^{1}H n.m.r. δ ($CDCl_3$) 1.80-2.35 (m, 4H), 3.78 (s, 3H), 7.35-7.45 (m, 3H), and 7.55-7.63 (m, 2H); ^{13}C n.m.r. δ ($CDCl_3$) 25.2, 29.3, 49.8 (t), 52.3, 59.3 (t), 127.3, 128.2, 128.4, 130.2, 169.7, and 172.8; $\upsilon_{max.}$ 1 745, 1 630 cm^{-1}; *m/z* 236 (M^+, 29%), 202 (3), 201 (2), 177 (10), 175 (30), and 174 (100). Deuterium content was established by mass spectrometry as 92% d_3; (Found; C, 65.94; H, 6.48; N, 5.80. Calc. for $C_{13}H_{12}NO_3D_3$: C, 66.08; H, 6.52; N, 5.93%). The value for the hydrogen content of the microanalysis was recalculated to allow for the 92% deuterium content at the 2- and 5-positions.

N-Benzoyl-5,5-dimethylproline methyl ester (111).

5,5-Dimethylproline (110) was prepared according to the method of

Bonnett *et. al.*[88] 4-Methyl-4-nitropentan-1-al (106) was prepared by condensation of 2-nitropropane (104) and acrolein (105) in the presence of sodium methoxide. Hence 2-nitropropane (104) (600 g, 6.7 mol) was added over 5 minutes at 10°C to a solution of sodium methoxide in methanol, prepared by the addition of sodium (25 g, 1.09 mol) to methanol (250 ml). The suspension was cooled to –10°C and acrolein (105) (63 g, 1.13 mol) in 2-nitropropane (104) (150 ml, 1.7 mol) added slowly over 2 h at 0°C. The suspension was warmed to room temperature and treated with 6M HCl until neutral to litmus. The solvent was removed *in vacuo* and the residue dissolved in benzene (300 ml), washed with water (3 x 700 ml), dried over anhydrous magnesium sulphate, filtered and concentrated to an oil. The oil was purified using a Kugelrohr apparatus to give 4-methyl-4-nitropentan-1-al (106), (43.07 g, 25%); b.p. 75°C / 1 mm Hg, Kugelrohr; ^{1}H n.m.r. δ ($CDCl_3$) 1.58 (s, 3H0, 2.3 (m, 4H), 8.35 (s, 1H). 4-Methyl-4-nitropentan-1-al (106) was used without further purification or characterisation. 4-Methyl-4-nitropentan-1-al (106) (43 g, 0.30 mol), dry ethylene glycol (19.5 ml, 0.32 mol) and toluene-*p* -sulphonic acid (0.63 g, 3.7 mmol) were heated at reflux in benzene (200 ml), the water produced being retained in a Dean-Stark apparatus. The benzene solution was washed with aqueous sodium hydrogen carbonate, dried over anhydrous magnesium sulphate, filtered and concentrated to an oil. The oil was purified using a Kugelrohr apparatus to give 2-(3-methyl-3-nitrobutyl)-1,3-dioxolan (107), (28.5 g, 51%); b.p. 100°C, 0.5 mm Hg, Kugelrohr (lit.,[88]105°C, 0.5 mm); ^{1}H n.m.r. δ (CCl_4) 1.40-2.18 (m, 10H), 3.80 (m, 4H), and 4.76 (t, J = 3 Hz, 1H); $\upsilon_{max.}$ (liquid film) 2 290, 2 920, 1 540, 1 480, 1 380, 1 370, 1 360, and 1 150 cm^{-1}. The dioxolan (107) (28.4 g, 0.15 mol) was hydrogenated in methanol (20 ml) over Raney nickel (3 g) at room temperature and 1800 psi for 24 h, the catalyst removed, the solvent evaporated *in vacuo* and the residue heated at reflux in 6M HCl (40 ml) for 30 minutes. The cooled solution was made alkaline with aqueous sodium hydroxide and extracted with ether (5 x 100 ml) to give the crude 5,5–dimethyl-Δ^1-pyrroline (108), (11 g, 75%); ^{1}H n.m.r. δ (CCl_4)

1.20 (s, 3H), 1.56 (m, 3H), 2.53 (m, 2H), and 7.22 (br. s, 1H); $\upsilon_{max.}$ (liquid film) 1 620, 1 375, 1 360, and 780 cm^{-1} (lit.,[88] 1 621, 1 378, and 1 361 cm^{-1}). To a stirred solution of potassium cyanide (10 g, 0.15 mol) and 5,5–dimethyl-Δ^1-pyrroline (108) (11 g, 0.11 mol) in water (44 ml) at 0°C dilute HCl (0.2 M, 110 ml) was added over 2 h. The solution was left to stand for 16 h at room temperature, then made alkaline with aqueous sodium hydroxide solution and extracted with ether. The extracts were combined, dried over anhydrous magnesium sulphate, filtered and concentrated to an oil. The oil was distilled to yield 5-cyano-2,2-dimethylpyrrolidine (109) (5.6 g, 40%); b.p. 162°C (lit.,[88] 164-166°C); ^{1}H n.m.r. δ (CCl_4) 1.20 (s, 3H), 1.34 (s, 3H), 1.35-2.40 (m, 4H), and 3.97 (m, 1H); $\upsilon_{max.}$ (liquid film) 3 340, 2 250, 1390, and 1 370 cm^{-1} (lit.,[88] 3 310, 2 245, 1 385, and 1 368 cm^{-1}). The 5-cyano-2,2-dimethylpyrrolidine (109) (5.6 g, 45 mmol) was heated at reflux in 6M HCl (20 ml) for 6 h. The solvent was removed *in vacuo* to give the crude 5,5-dimethylproline hydrochloride (110) which was derivitised without purification or characterisation.

N-Benzoyl-5,5-dimethylproline methyl ester (111) was prepared from the crude 5,5-dimethylproline hydrochloride (110) as described above for N-benzoyl-(2S)-proline methyl ester (100a). The crude N-benzoyl-5,5-dimethylproline methyl ester (111) was distilled under reduced pressure and further purified by preparative scale h.p.l.c. using a Lobar "LiChroprep" cyanopropyl column (25 cm x 2.5 cm i.d.) with hexane / propan-2-ol (19:1) as eluant to give N-benzoyl-5,5-dimethylproline methyl ester (111) as an oil, yield (based on 5-cyano-2,2-dimethylpyrrolidine (109)) 35%; ^{1}H n.m.r. δ ($CDCl_3$) 1.58 (s, 3H), 1.72 (s, 3H), 1.80-2.20 (m, 4H), 3.47 (s, 3H), 4.28 (br. d, J = 7 Hz, 1H), and 7.27-7.39 (m, 5H); ^{13}C n.m.r. δ ($CDCl_3$) 25.1, 25.5, 25.6, 27.6, 39.7, 57.6, 63.1, 125.4, 127.4, 128.3, 128.6, 169.8, and 172.6; $\upsilon_{max.}$ 2 980, 1 750, 1 640, 1 390, 1 370, 1 205, and 1 105 cm^{-1}; *m/z* 261.1367 (M^+) [Calc. for $C_{15}H_{19}NO_3$ (M^+) *m/z* 261.1365] (Found: C, 68.58; H, 7.58; N, 5.37. Calc. for $C_{15}H_{19}NO_3$: C, 68.48; H, 7.61; N, 5.51%).

Raney Nickel.[108]

Nickel-aluminium alloy (6 g) was carefully added to a cooled, stirred solution of sodium hydroxide (76 g, 300 ml). The contents were allowed to come to room temperature and were left to stand for 1 hour, followed by heating on a steam bath for 10 h. The contents were washed with distilled water (3 x 50 ml), an aqueous solution of sodium hydroxide (10% w/v) and distilled water until the washings were neutral to litmus. The contents were washed with anhydrous methanol (6 x 50 ml) and stored under anhydrous methanol.

Reaction of N-benzoyl-(2S)-proline methyl ester (100a) with NBS.

A mixture of N-benzoyl-(2S)-proline methyl ester (100a) (0.5 g, 2.1 mmol) and N-bromosuccinimide (1.14 g, 6.4 mmol) in carbon tetrachloride (80 ml), was heated at reflux, while irradiated with a 250 W mercury lamp, under nitrogen, for 1 hour. The suspension was chilled in an ice / salt bath for 1 hour, filtered and the solvent removed *in vacuo* . The crude reaction mixtures were analysed by h.p.l.c. using a Du Pont cyanopropyl column eluting with hexane / 2-propanol to determine the product ratios. Complete reaction afforded N-benzoyl-2-methoxycarbonyl-4-bromopyrrole (113) and N-benzoyl-2-methoxycarbonyl-3-bromopyrrole (114) in the ratio of 3.3 : 1.0. Reaction with less than 3 equivalents of NBS gave incomplete reaction of the proline derivative. In reaction mixtures where less than 3 equivalents of NBS were used N-benzoyl-2-methoxycarbonyl-pyrrole (115) was observed by h.p.l.c. and ^{1}H and ^{13}C n.m.r. spectroscopy. Attempts to fully characterise (115) were unsuccessful. Chromatography using the Chromatotron afforded N-benzoyl-2-methoxycarbonyl-4-bromopyrrole (113), (96 mg, 14%), m.p. 88-90°C; ^{1}H n.m.r. δ ($CDCl_3$) 3.60 (s, 3H), 7.03 (d, J = 2 Hz, 1H), 7.22 (d, J = 2 Hz, 1H), and 7.40-7.90 (m, 5H); ^{13}C n.m.r. δ ($CDCl_3$) 51.9, 99.3, 122.5, 126.4, 126.6, 128.9, 130.0, 132.6, 134.0, 159.7, and 167.1; $\upsilon_{max.}$ 1 715, 1 260, 710, and 690 cm^{-1}; *m/z* 309, 307 (M^+,92 and 96% respectively), 278

(5), 276 (6), 228 (10), 205 (10), 203 (9), and 176 (100); *m/z* 306.9843 (M^+) [Calc. for $C_{13}H_{10}NO_3Br$ (M^+) *m/z* 306.9845] (Found: C, 50 83; H, 3.05; N, 4.31. Calc. for $C_{13}H_{10}NO_3Br$: C, 50.86; H, 3.26; N, 4.56%), and N-benzoyl-2-methoxy-carbonyl-3-bromopyrrole (114), (190 mg, 29%); ^1H n.m.r. δ ($CDCl_3$) 3.57 (s, 3H), 6.39 (d, J = 4 Hz, 1H), 7.14 (d, J = 4 Hz, 1H), 7.45-7.53 (m, 3H), and 7.73-7.77 (m, 2H); ^{13}C n.m.r. δ ($CDCl_3$) 51.9, 109.4, 114.9, 123.8, 126.3, 128.9, 129.8, 132.5, 133.8, 160.0, and 167.1; *m/z* 309, 307 (M^+, 35 and 36% respectively), 278 (3), 276 (3), 251 (3), 249 (3), and 230 (100); $\upsilon_{max.}$ (liquid film) 3 080, 2 995, 1 720, 1 710, and 720; *m/z* 306.9840 (M^+) [Calc. for $C_{13}H_{10}NO_3Br$ (M^+) *m/z* 306.9845]. Also observed was N-benzoyl-2-methoxycarbonylpyrrole (115) ^1H n.m.r. δ ($CDCl_3$) 3.58, (s, 3H), 6.31 (t, J = 3 Hz, 1H), 7.07 (dd, J = 2 Hz, J = 3 Hz, 1H), 7.23 (dd, J = 2 Hz, J = 3 Hz, 1H), 7.59-7.66 (m, 3H), and 7.73-7.77 (m, 2H); ^{13}C n.m.r. δ ($CDCl_3$) 51.6, 110.6, 121.3, 126.0, 127.7, 128.7, 129.9, 133.4, 133.6, 160.7, and 168.3.

Measurement of the relative rates of reaction of the amino acid derivatives (29a), (40a) and (93a), and their deuterated analogues (29c), (40b) and (93b), with NBS.

The relative rates of reaction of the compounds were determined in competive experiments. Typically a mixture of N-benzoylalanine methyl ester (40a) (50 mg, 0.24 mmol) and N-benzoylglycine methyl ester (93a) (50 mg, 0.26 mmol), with *t*-butylbenzamide (57) (50 mg, 0.28 mmol) as an internal standard, in carbon tetrachloride (10 ml), was treated with NBS (50 mg, 0.28 mmol). The mixture was heated at reflux, while irradiated with a 250 W mercury lamp, under nitrogen, for 30 minutes. The starting and product mixtures were analysed by h.p.l.c. using a Du Pont Zorbax cyanopropyl column. The initial and final ratios of the amino acid derivatives (40a) and (93a) were determined by integration of the h.p.l.c. trace by a Hewlett Packard HP3390A integrator. The areas of the two substrates in the product mixtures were corrected using the internal standard by the factor $Area(STD_{SM}) / Area(STD_{Sample})$. The peak areas in the starting

material and product mixtures were normalised by a factor of 100% / $Area_{SM}$. This converts the peak areas to % unreacted substrate. The relative rates of reaction were calculated using equation (14) and are shown in Table 17. The results were averaged and the error shown represents the spread of results. The error in the integration of the peaks is assumed to be negligible.

The relative rates of reaction of the other amino acid derivatives were measured similarly. No allowance was made for incomplete deuterium encorporation in calculating the deuterium isotope effects.

Table 17a: Relative Rates of Reaction of (40a) and (93a) with NBS.

	Sample				
	1	2	3	4	5
$k_{(93a)} / k_{(40a)}$	2.90	3.04	2.97	3.10	3.01

$k_{(93a)} / k_{(40a)} = 3.0 \pm 0.1$

Table 17b:Relative Rates of Reaction of (40a) and (93b) with NBS.

	Sample				
	1	2	3	4	5
$k_{(93b)} / k_{(40a)}$	0.89	0.97	0.96	0.95	0.91
	6	7	8	9	
	0.92	0.94	0.92		

$k_{(93b)} / k_{(40a)} = 0.95 \pm 0.06$

Table 17c:Relative Rates of Reaction of (40b) and (29a) with NBS.

	Sample			
	1	2	3	4
$k_{(40b)} / k_{(29a)}$	4.03	3.79	4.48	4.28

$$k_{(40b)} / k_{(29a)} = 4.1 \pm 0.4$$

Table 17d: Relative Rates of Reaction of (40b) and (93a) with NBS.

	Sample			
	1	2	3	4
$k_{(40b)} / k_{(93a)}$	6.10	5.55	5.32	5.53

$$k_{(40b)} / k_{(93a)} = 5.6 \pm 0.5$$

Table 17e: Relative Rates of Reaction of (40b) and (29c) with NBS.

	Sample			
	1	2	3	4
$k_{(40b)} / k_{(29c)}$	16.2	10.4	17.6	21.5

$$k_{(40b)} / k_{(29c)} = 16 \pm 6$$

Measurement of the relative rates of reaction of the amino acid derivatives (23a) and (100a), and their deuterated analogues (23b), (100b) and (100c), with NBS.

The rates of reaction of the amino acid derivatives (23a) and (100a), and their deuterated analogues (23b), (100b) and (100c), were measured in competitive experiments, relative to the alanine and glycine derivatives (40a) and (93a) as described above. Analyses of reaction mixtures containing methyl pyroglutamate (23a) or methyl [2-^{2}H]-pyroglutamate (23b) were carried out by g.l.c. using a 2% Carbowax 20M capillary column. Analyses of reaction mixtures containing the proline derivatives (100a-c) were carried out by h.p.l.c. as described above. The relative rates of reaction were calculated using equation (14) and are shown in Table 18. The results were averaged and the error shown represents the spread of results. No allowance was made for incomplete

deuterium encorporation in calculating the deuterium isotope effects.

Table 18a: Relative Rates of Reaction of (23a) and (93a) with NBS.

	Sample			
	1	2	3	4
$k_{(23a)} / k_{(93a)}$	2.95	2.90	2.92	2.75
	5	6	7	8
	3.02	2.89	3.80	3.83

$k_{(23a)} / k_{(93a)} = 3.1 \pm 0.7$

Table 18b: Relative Rates of Reaction of (23b) and (93a) with NBS.

	Sample					
	1	2	3	4	5	6
$k_{(23b)} / k_{(93a)}$	2.05	1.78	2.38	1.80	2.42	2.30

$k_{(23b)} / k_{(93a)} = 2.1 \pm 0.3$

Table 18c: Relative Rates of Reaction of (93a) and (100a) with NBS.

	Sample			
	1	2	3	4
$k_{(100a)} / k_{(93a)}$	1.38	1.38	1.34	1.37
	5	6	7	
	1.43	1.43	1.51	

$k_{(100a)} / k_{(93a)} = 1.41 \pm 0.1$

Table 18e: Relative Rates of Reaction of (93a) and (100b) with NBS.

	Sample					
	1	2	3	4	5	6
$k_{(100b)} / k_{(93a)}$	1.21	1.08	1.13	1.15	1.16	1.25

$k_{(100b)} / k_{(93a)} = 1.2 \pm 0.1$

Table 18f: Relative Rates of Reaction of (40a) and (100c) with NBS.

	Sample					
	1	2	3	4	5	6
$k_{(40a)} / k_{(100c)}$	0.84	0.80	0.77	0.77	0.83	0.75

$k_{(40a)} / k_{(100c)} = 0.79 \pm 0.04$

N-Phthaloylvaline methyl ester (122).

Valine (49a) (6 g, 51 mmol) was treated with sodium carbonate solution (2 M, 100 ml) and N-phthaloyl acetate (13.5 g, 67 mmol). The suspension was stirred at room temperature for 2 h, then treated with HCl (6M, 60 ml). The suspension was filtered, the filtrate extracted with ethyl acetate (4 x 80 ml), the extracts combined, dried over anhydrous magnesium sulphate, filtered and the solvent removed *in vacuo* . The resulting oil was dissolved in methanol (50 ml) to which thionyl chloride (7 ml) had been added, and the solution was left to stand at room temperature for 16 h. Concentration of the solution and chromatography afforded N-phthaloylvaline methyl ester (122) which crystallised from ether / pet. ether, (5.5 g, 41%), m.p. 37-38°C (lit.,[93] 39-41°C); ^{1}H n.m.r. δ ($CDCl_3$) 0.91 (d, J = 6 Hz, 3H), 1.15 (d, J = 6 Hz, 3H), 2.80 (m, 1H), 3.91 (s, 3H), 4.60 (d, J = 7 Hz, 1H), and 7.55-8.05 (m, 4H).

N-Phthaloyl-3-bromovaline methyl ester (123).

A mixture of N-phthaloylvaline methyl ester (122) (0.75 g, 2.9 mmol) and N-bromosuccinimide (0.6 g, 3.4 mmol), in carbon tetrachloride (30 ml), was heated at reflux, while irradiated with a 250 W mercury lamp, under nitrogen, for 1 hour. The cooled suspension was filtered and the solvent removed *in vacuo* to give the crude N-phthaloyl-3-bromovaline methyl ester (123) in quantitative yield. Successive recrystallisations from carbon tetrachloride / hexane gave the pure compound, (0.25 g, 26%); m.p. 129-131°C; ^{1}H n.m.r. δ ($CDCl_3$) 2.00 (s, 3H), 2.16 (s, 3H), 3.71 (s, 1H), 5.18 (s, 1H), and 7.40-8.05 (m, 4H); ^{13}C n.m.r. δ ($CDCl_3$) 31.8, 32.7, 52.6, 60.1, 64.4, 123.9, 131.6, 134.5, 166.1, and 167.3; $\upsilon_{max.}$ 1 750, 1 720, 1 380, and 715; *m/z* 341, 339 (M^+, 4 and 5% respectively), 310 (4), 308 (4), and 260 (100); *m/z* 339.0102 (M+) [Calc. for $C_{14}H_{14}BrNO_4$ (M+) *m/z* 339.0107] (Found: C, 49.07; H, 4.42; N, 4.08. Calc. for $C_{14}H_{14}BrNO_4$: C, 49.43; H, 4.15; N, 4.12%).

Triphenyltin deuteride.[66]

Triphenyltin deuteride was prepared as described above for triphenyltin hydride from triphenyltin chloride and lithium aluminium hydride.

N-Benzoyl-[2-^{2}H]-glycine methyl ester (93c).

N-Benzoyl-2-bromoglycine methyl ester (112a), prepared from N-benzoylglycine methyl ester (93a) (0.5 g, 2.6 mmol) as described above, was dissolved in benzene (20 ml), treated with triphenyltin deuteride (1.8 g, 5 mmol) and left to stand overnight. The solvent was removed *in vacuo* and the residue chromatographed on silica with ethyl acetate / dichloromethane (1:19) as eluant. Recrystallisation from ethyl acetate / pet. ether gave N-benzoyl-[2-^{2}H]-glycine methyl ester (93c), (0.31 g, 62%), m.p. 77-78°C (lit.,[104] 82-83°C); ^{1}H n.m.r. δ ($CDCl_3$) 3.81 (s, 3H), 4.22-4.25 (m, 1H), 6.67 (br. m, 1H), 7.42-7.55 (m, 3H), and 7.81-7.83 (m, 2H); ^{13}C n.m.r. δ ($CDCl_3$) 41.4 (t, J = 21 Hz), 52.4, 126.9, 128.4, 131.6, 133.5, 167.2, and 170.4; *m/z* 194 (M^+, 50%), 163 (6), 162 (8), 161 (7), 135 (51), and 105 (100). Deuterium content was established by mass spectrometry as 97%.

N-Benzoylglycylvaline methyl ester (126a).

(2S)-Valine (49a) (Sigma, 0.8 g, 6.8 mmol) was added to anhydrous methanol (20 ml) to which thionyl chloride (4 ml, 54 mmol) had been added and the solution left to stand for 16 h. The solvent was removed *in vacuo* to give the crude (2S)-valine methyl ester hydrochloride (72). Valine methyl ester hydrochloride (72) and N-methylmorpholine (0.75 ml, 7.4 mmol) were added to a stirred and cooled solution of hippuric acid (45) (BDH, 1.2 g, 6.7 mmol), dicyclohexylcarbodiimide (1.44 g, 7.4 mmol), and N-methylmorpholine (0.75 ml, 7.4 mmol) in dichloromethane (10 ml). The mixture was stirred at 0°C for 2 h and

then allowed to come to room temperature. After standing for 2 h the mixture was filtered, washed with dichloromethane (2 x 20 ml) and the combined filtrate and washes washed with aqueous sodium bicarbonate (1% w / v, 2 x 10 ml), dried over anhydrous magnesium sulphate, filtered and the solvent removed *in vacuo* . N-Benzoylglycylvaline methyl ester (126a) was recrystallised from ethyl acetate / pet. ether (0.24 g, 12%), m.p. 137-138°C (lit.,[109] 136-138°C); ^{1}H n.m.r. δ ($CDCl_3$) 0.91 (d, J = 6 Hz, 3H), 0.94 (d, J = 6 Hz, 3H), 2.2 (m, 1H), 3.70 (s, 3H), 4.22 (d, J = 4 Hz, 2H), 4.50 (dd, J = 4 Hz, J = 8 Hz, 1H), and 7.1-8.0 (m, 7H); *m/z* 292 (M^+, 4%), 233 (4), 224 (2), 194 (10), 176 (8), 163 (14), 162 (7), 161 (14), 135 (17), 134 (33), 133 (62), and 105 (100).

N-Benzoyl-[2-^{2}H]-glycylvaline methyl ester (126b).

A mixture of N-benzoylglycylvaline methyl ester (126a) (0.21 g, 0.72 mmol) and NBS (0.12 g, 0.67 mmol) in carbon tetrachloride (40 ml) was heated at reflux, while irradiated with a 250 W mercury lamp, under nitrogen, for 60 minutes. The mixture was chilled in an ice / salt bath for 30 minutes, filtered and the solvent removed *in vacuo* to give the crude N-benzoyl-2-bromoglycylvaline methyl ester (127), ^{1}H n.m.r. δ ($CDCl_3$) 0.90 (d, J = 6 Hz, 3H), 0.94 (d, J = 6 Hz, 3H), 2.2 (m, 1H), 3.72 (s, 3H), 4.50 (dd, J = 4 Hz, J = 8 Hz, 1H), 6.95 (d, J = 8 Hz, 1H), and 7.20-8.20 (m, 7H). This was dissolved in benzene (10 ml), treated with triphenyltin deuteride (0.91 g, 2.6 mmol) and left to stand for 16 h. The solvent was removed *in vacuo* and the mixture purified by column chromatography on silica with ethyl acetate / dichloromethane (1:19) as eluant. N-Benzoyl-[2-^{2}H]-glycylvaline methyl ester (126b) was recrystallised from ethyl acetate / pet. ether, (56 mg, 27%); m.p. 124-126°C (lit.,[109] 136-138°C); ^{1}H n.m.r. δ ($CDCl_3$) 0.91 (d, J = 6 Hz, 3H), 0.94 (d, J = 6 Hz, 3H), 2.2 (m, 1H), 3.72 (s, 3H), 4.22 (d, J = 4 Hz, 1H), 4.52 (dd, J = 4 Hz, J = 8 Hz, 1H), 6.65 (br. s, 1H), 7.15 (br. d, J = 8 Hz, 1H), 7.305-7.60 (m, 3H), and 7.75-7.90 (m, 2H); ^{13}C n.m.r. δ ($CDCl_3$) 17.8, 19.0, 31.0, 43.5 (t, J = 16 Hz), 52.2, 57.6, 127.0, 128.5, 132.3, 133.1, 167.9, 169.4, 171.8;

m/z 293 (M^+, 20%), 234 (19), 180 (15), 163 (40), 136 (65), and 135 (100). Deuterium content was established by mass spectrometry as 82%.

Also isolated were the two diastereoisomers of N-benzoyl-2-ethoxy-glycylvaline methyl ester (128); (i) (20 mg, 8%), ^{1}H n.m.r. δ ($CDCl_3$) 0.92 (d, J = 6 Hz, 3H), 0.94 (d, J = 6 Hz, 3H), 1.31 (t, J = 7 Hz, 3H), 2.2 (m, 1H), 3.77 (s, 3H), 3.84 (q, J = 7 Hz, 2H), 4.54 (dd, J = 5 Hz, J = 9 Hz, 1H), 5.92 (d, J = 9 Hz, 1H), 7.41-7.56 (m, 5H), and 7.84-7.87 (m, 2H); ^{13}C n.m.r. δ ($CDCl_3$) 15.0, 17.7, 18.9, 31.2, 52.3, 57.5, 64.7, 77.8, 127.2, 128.5, 132.1, 132.7, 168.3, 168.9, and 171.4; *m/z* 336 (M^+,20%), 307 (26), 291 (100), and 290 (68); *m/z* 336.1691 (M) [Calc. for $C_{17}H_{24}N_2O_5$ (M^+) *m/z* 336.1685], and (ii), (18 mg, 8%);^{1}H n.m.r. δ ($CDCl_3$) 0.93 (d, J = 6 Hz, 3H), 0.98 (d, J = 6 Hz, 3H), 1.30 (t, J = 7 Hz, 3H), 2.2 (m, 1H), 3.74 (s, 3H), 3.84 (q, J = 7 Hz, 2H), 4.54 (dd, J = 5 Hz, J = 9 Hz, 1H), 5.82 (d, J = 8 Hz, 1H), 7.30-7.56 (m, 5H), and 7.84-7.87 (m, 2H); ^{13}C n.m.r. δ ($CDCl_3$) 15.3, 17.8, 19.1, 31.3, 52.4, 57.4, 64.6, 78.0, 127.2, 128.6, 132.1, 132.7, 168.3, 169.4, and 171.5; *m/z* 336 (M^+,24%), 307 (31), 291 (92), 290 (93), and 259 (100); *m/z* 336.1701 (M) [Calc. for $C_{17}H_{24}N_2O_5$ (M^+) *m/z* 336.1685].

Reaction of N-benzoyl-2-bromoglycine methyl ester (112a) with $(nBu_3Sn)_2$.

N-Benzoyl-2-bromoglycine methyl ester (112a) was prepared from N-benzoylglycine methyl ester (93a) (2.0 g, 10 mmol) as described above and dissolved in benzene (200 ml). Hexabutylditin (8.0 g, 11 mmol) was added and the solution heated at reflux, while irradiated with a 250 W mercury lamp, under nitrogen, for 2 h. The solution was cooled to room temperature and washed with saturated aqueous sodium fluoride (4 x 250 ml). The organic layer was dried over anhydrous magnesium sulphate, filtered and the solvent removed *in vacuo*. The residue was washed with pet. ether (200 ml), the pet. ether fraction decanted and any remaining solvent removed *in vacuo*. Integration of the ^{1}H n.m.r. spectrum of the residue was used to calculate the relative yields of the compounds (131-4) which are presented in Table 19. Chromatography using

the Chromatron afforded the following products. The order of elution was (134), (131), (132), and (133).

Dimethyl 2,3-benzamidobutane-1,4-dioate (131a).

(47 mg, 1.2%), m.p. 181-182°C; ^{1}H n.m.r. δ ($CDCl_3$) 3.84 (s, 6H)), 5.34 (d, J = 6 Hz, 2H), 7.26-7.59 (m, 6H), 7.89-7.91 (m, 4H), and 7.98 (br. d, J = 6 Hz, 2H); ^{13}C n.m.r. δ ($CDCl_3$) 53.2, 56.2, 127.4, 128.7, 132.2, 132.9, 168.2, and 169.1; $\upsilon_{max.}$ 3 420, 1 740, 1 640, and 1 540 cm^{-1}; *m/z* 384.1301 (M^+) [Calc. for $C_{20}H_{20}N_2O_6$ (M^+) *m/z* 384.1321] (Found: C, 62.72; H, 5.14; N, 7.33. Calc. for $C_{20}H_{20}N_2O_6$: C, 62.49; H, 5.24; N, 7.29%).

Dimethyl 2,3-benzamidobutane-1,4-dioate (131b).

(43 mg, 3.5%), m.p. 206-207°C; ^{1}H n.m.r. δ ($CDCl_3$) 3.89 (s, 6H), 5.33 (d, J = 7 Hz, 2H), 7.15 (br. d, J = 7 Hz, 2H), 7.40-7.56 (m, 6H), and 7.78-7.82 (m, 4H); ^{13}C n.m.r. δ ($CDCl_3$) 53.4, 54.5, 127.4, 128.7, 132.1, 133.2, 167.4, and 170.3; $\upsilon_{max.}$ 3 350, 1 745, 1 635, and 1 540 cm^{-1}; *m/z* 263.0805 (M^+-$PhCONH_2$) [Calc. for $C_{13}H_{13}NO_5$ (M^+- $PhCONH_2$) *m/z* 263.0794] (Found: C, 62.19; H, 5.14; N, 7.22. Calc. for $C_{20}H_{20}N_2O_6$: C, 62.49; H,5.24; N, 7.29%).

Dimethyl 2,4-benzamido-3-oxypentane-1,5-dioate (132a).

(0.45 g, 10.9%), m.p. 154-156°C; ^{1}H n.m.r. δ ($CDCl_3$) 3.77 (s, 6H), 6.22 (d, J = 8 Hz, 2H), 7.40-7.42 (m, 6H), 7.73 (br. d, J = 8 Hz, 2H), and 7.83-7.87 (m, 4H); ^{13}C n.m.r. δ ($CDCl_3$) 53.2, 76.5, 127.5, 128.7, 132.4, 132.7, 167.4, and 168.0; $\upsilon_{max.}$ 3 410, 1 765, 1 655, 1 540, 1 080 cm^{-1}; (Found: C, 53.57; H, 4.48; N, 6.14. Calc. for $(C_{20}H_{20}N_2O_7)_2.CHCl_3$: C, 53.51; H, 4.49; N, 6.09%). The crystal structure data is tabulated in Appendix 1.

Dimethyl 2,4-benzamido-3-oxypentane-1,5-dioate (132b).

(0.20 g, 4.8%), m.p. 128-129°C; ^{1}H n.m.r. δ ($CDCl_3$) 3.81 (s, 6H), 6.17 (d, J = 8 Hz, 2H), 7.42-7.59 (m, 6H), 7.72 (br. d, J = 8 Hz, 2H), and 7.91-7.95 (m, 4H); ^{13}C n.m.r. δ ($CDCl_3$) 53.3, 75.4, 127.5, 128.7, 133.0, 132.8, 167.5, and 168.1; $\upsilon_{max.}$ 3 310, 1 760, 1 540, 1 095 cm^{-1}; (Found: C. 59.99; H, 5.09; N, 7.01. Calc. for $C_{20}H_{20}N_2O_7$: C, 59.99; H, 5.04; N, 7.01%).

N-Benzoyl-2-hydroxyglycine methyl ester (133).

(176 mg, 8.1%), m.p. 108-110°C (lit.,[110] m.p. 117-118°C); ^{1}H n.m.r. δ ($CDCl_3$) 3.88 (s, 3H), 5.19 (d, J = 6 Hz, 1H), 5.83 (t, J = 6 Hz, 1H), 7.43-7.58 (m, 3H), 7.76 (br. d, J = 6 Hz, 1H), 7.81- 7.84 (m, 2H); ^{13}C n.m.r. δ ($CDCl_3$) 53.4, 72.7, 127.3, 128.7, 132.5, 132.7, 168.0, 169.0; $\upsilon_{max.}$ 3 650, 3 420, 1 760, 1 545 cm^{-1}; *m/z* 208.0614 (M^+-H) [Calc. for $C_{10}H_{10}NO_4$ (M^+-H) *m/z* 208.0610] (Found: C, 57.71; H, 5.24; N, 6.65. Calc. for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30; N, 6.69%).

N-Benzoyl-2-benzamidoglycine methyl ester (134).

(239 mg, 7.4%), m.p. 204-206°C; ^{1}H n.m.r. δ ($CDCl_3$) 3.86 (s, 3H), 5.91 (t, J = 6 Hz, 1H), 7.40-7.56 (m, 6H), 7.82-7.86 (m, 4H), 7.95 (d, J = 6 Hz, 2H); ^{13}C n.m.r. δ ($CDCl_3$) 53.5, 58.0, 127.4, 128.7, 132.3, 132.7, 167.7, 168.6; $\upsilon_{max.}$ 3 400, 1 760, 1 645, 1 540 cm^{-1}; *m/z* 253.0975 ($(PhCONH)_2CH^+$) [Calc. for $C_{15}H_{13}N_2CH^+$ ($(PhCONH)_2CH^+$) 253.0977].

A number of products totalling 3% of the crude mixture were not isolated. Also isolated was N-benzoyl-2-ethoxyglycine methyl ester (135), (43 mg, 2%); m.p. 65-66°C; ^{1}H n.m.r. δ ($CDCl_3$) 1.25 (t, J = 8 Hz, 3H), 3.78 (q, J = 8 Hz, 2H), 3.84 (s, 3H), 5.86 (d, J = 9 Hz, 1H), 7.22 (br. d, J = 9 Hz, 1H), 7.44-7.59 (m, 3H), and 7.84-7.87 (m, 2H); ^{13}C n.m.r. δ ($CDCl_3$) 15.9, 52.9, 65.3, 77.4, 127.3, 128.7, 132.3, 133.2, 167.2, and 168.8; $\upsilon_{max.}$ 3 460, 1 765, 1 665, and 1 535 cm^{-1}; (Found C, 60.96; H, 6.48; N, 5.91. Calc. for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N,

5.91%).

The experiment was repeated under more stringently oxygen-free conditions. The solution of N-benzoyl-2-bromoglycine methyl ester (112a) and hexabutylditin in benzene was heated at reflux and then cooled in an ice / salt bath while being purged with nitrogen. This was repeated and the solution heated at reflux, while irradiated with a 250 W mercury lamp, under nitrogen, for 2 h. The solution was cooled, washed with saturated aqueous sodium flouride (4 x 250 ml), dried over anhydrous magnesium sulphate, filtered and the solvent removed *in vacuo* . The residue was washed with pet. ether (200 ml), the pet. ether decanted and remaining solvent removed *in vacuo* to give the crude product mixture. Analysis by ^{1}H n.m.r. spectroscopy was used to calculate the relative yields of the compounds. The results are tabulated in Table 19.

Table 19.

	Experiment 1.		Experiment 2.	
Compound.	Relative Yield[a]	Actual Yield[b]	Relative Yield[a]	Actual Yield[b]
(131a)	5	47 mg, 1.2%	15%	144 mg, 3.6%
(131b)	5	43 mg, 1.1%	15%	80 mg, 2.0%
(132a)	30	450 mg, 10.9%	23%	--
(132b)	30	200 mg, 4.8%	23%	--
(133)	10	176 mg, 8.1%	14%	--
(134)	17	239 mg, 7.4%	15%	--

[a]Analysis by ^{1}H n.m.r. spectroscopy.

[b]Based on N-benzoylglycine methyl ester (112a).

APPENDIX.

X-RAY CRYSTAL STRUCTURE OF ETHER (132A).

Structure Determination.

Table 20 lists the crystal data and X-ray experimental details for the structure determination. Intensity data were collected with a Nicolet R3m four-circle diffractometer using monochromated Mo Kα radiation. Cell parameters were determined by least-squares refinement, the setting angles of 25 accurately centred reflections ($2\theta > 20°$) being used. The intensities of three standard reflections (300, 060, 009) were monitored throughout data collection and this indicated no significant crystal decomposition. Intensities were corrected for Lorentz and polarization effects but no correction was made for absorption.

The structure was solved by direct methods and refined by blocked cascade least-squares procedures. The asymmetric unit contains two independent molecules of the ether and a molecule of chloroform which is disordered over two orientations with relative site occupancies of 0.75 and 0.25. Except for the carbon of the minor chloroform contributor all non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were included in calculated positions with isotropic thermal parameters equal to the isotropic equivalent of their carrier atoms. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$, with $w = [\sigma^2(F_o) + gF_o^2]^{-1}$. All calculations were performed on Nova 4X or DG30 computers using SHELXTL.[111] Table 21 lists the final coordinates for the non-hydrogen atoms with estimated standard deviations in parentheses.

Discussion of the Structure

The structure of the ether is shown to be that of the d,l-diastereoisomer. The asymmetric unit contains two molecules of the ether and a disordered chloroform molecule. Figure 6 shows perspective views and atom labelling of the two independent molecules viewed in similar orientations (and both arbitrarily

chosen as the R,R-enantiomer). Comparable bond lengths (Table 22) and bond angles (Table 23) are similar within the two halves of each molecule and between the two independent molecules. These bond lengths and angles are similar to those found in structurally related compounds.[95]

However, significant torsional angels differences exist within the two halves of each molecule. For example the two hydrogens attached to C(10) and N(10) in molecule A are almost eclipsed (i.e. syn-coplanar) [H-N-C-H = 13.3(2)°] whilst the hydrogens attached to C(20) and N(20) are approximately anti-coplanar [H-N-C-H = 177.5(1)°]. Similarly conformational differences exist between the two molecules. For example the meanplanes through the two phenyl rings in molecule A are mutually inclined at an angle of 12.6(2)° whilst the corresponding value for molecule B is 30.5(2)°. These torsional differences are related to the molecular packing which is controlled by a complex network of hydrogen bonds. As listed in Table 24 all four independent amide hydrogens are hydrogen bonded to carbonyl oxygens of adjacent molecules.

Table 20. Crystal Data and X-Ray Experimental Details

Formula	$C_{20}H_{20}N_2O_7.{}^1/_2(CHCl_3)$
Molecular Weight	460.1
Crystal System	triclinic
Space Group	P1
a (Å)	10.010(3)
b (Å)	13.254(4)
c (Å)	17.154(6)
α (°)	74.83(3)
β (°)	80.73(3)
γ (°)	81.76(2)
V ($Å^3$)	2156(1)
Dc ($g\ cm^{-3}$)	1.417
Z	4
F (000)	956
μ (cm^{-1})	2.8
Radiation	Mo Kα
Wavelength (Å)	0.71069
Temperature (°C)	-130
Crystal dimensions (mm)	0.58 x 0.26 x 0.20
Scan mode	ω
2θ range (°)	3 - 50
Unique reflections	7312
Observed reflections ($1 > 3\sigma(I)$)	4692
Number of parameters	590
g	0.00054
R	0.060
wR	0.076

Table 21. Atom coordinates ($x10^4$) and temperature factors ($Å^2 x 10^3$)

	Molecule A					Molecule B			
atom	x	y	z	U_{eq}[a]	atom	x	y	z	U_{eq}[a]
O(10)	9229(3)	6345(2)	4674(2)	26(1)	O(30)	-613(3)	8684(2)	534(2)	23(1)
O(11)	10362(3)	7002(2)	5741(2)	36(1)	O(31)	-3066(3)	9658(2)	328(2)	31(1)
O(12)	10766(3)	8512(2)	4820(2)	35(1)	O(32)	-4008(3)	8673(2)	1505(2)	34(1)
O(13)	7656(3)	8362(2)	5036(2)	35(1)	O(33)	-2823(3)	7269(2)	383(2)	37(1)
N(10)	9000(3)	8112(2)	3904(2)	27(1)	N(30)	-1671(3)	7248(2)	1419(2)	26(1)
C(10)	9867(4)	7280(3)	4368(2)	26(1)	C(30)	-1641(4)	8369(3)	1195(2)	25(1)
C(11)	10314(4)	7573(3)	5073(2)	27(1)	C(31)	-2978(4)	8967(3)	934(2)	25(1)
C(12)	11434(5)	8851(3)	5381(3)	43(2)	C(32)	-5335(4)	9206(4)	1339(3)	44(2)
C(13)	7923(4)	8614(3)	4293(2)	28(1)	C(33)	-2189(4)	6765(3)	940(3)	31(2)
C(14)	7073(4)	9455(3)	3769(2)	28(1)	C(34)	-1927(4)	5595(3)	1130(3)	31(2)
C(15)	5742(5)	9699(4)	4082(3)	41(2)	C(35)	-1913(5)	5116(4)	502(3)	47(2)
C(16)	4889(5)	10459(4)	3618(3)	48(2)	C(36)	-1611(6)	4038(4)	633(3)	57(2)
C(17)	5367(5)	10958(4)	2844(3)	42(2)	C(37)	-1334(5)	3437(4)	1390(3)	50(2)
C(18)	6690(5)	10734(3)	2527(3)	40(2)	C(38)	-1367(5)	3906(3)	2016(3)	45(2)
C(19)	7543(5)	9976(3)	2987(3)	35(2)	C(39)	-1655(4)	4980(3)	1891(3)	36(2)
O(21)	7613(3)	4797(2)	5129(2)	37(1)	O(41)	1527(3)	8597(2)	-630(2)	34(1)
O(22)	7550(3)	4838(2)	3816(2)	38(1)	O(42)	2887(3)	7873(2)	339(2)	32(1)
O(23)	10366(3)	6355(2)	2462(2)	35(1)	O(43)	523(3)	8741(2)	2349(2)	34(1)
N(20)	10295(3)	5343(2)	3743(2)	27(1)	N(40)	1016(3)	9438(2)	1012(2)	24(1)
C(20)	9049(4)	5872(3)	4052(2)	25(1)	C(40)	705(4)	8534(3)	784(2)	24(1)
C(21)	7992(4)	5107(3)	4417(3)	29(1)	C(41)	1726(4)	8349(3)	62(2)	25(1)
C(22)	6538(5)	4107(4)	4063(3)	52(2)	C(42)	4016(4)	7740(4)	-276(3)	41(2)
C(23)	10884(4)	5627(3)	2963(2)	28(1)	C(43)	912(4)	9476(3)	1799(2)	23(1)
C(24)	12187(4)	5007(3)	2743(3)	30(2)	C(44)	1304(4)	10436(3)	1964(2)	27(1)
C(25)	12789(4)	4159(3)	3279(3)	33(2)	C(45)	1711(4)	11291(3)	1359(3)	34(2)
C(26)	14015(5)	3631(4)	3032(3)	45(2)	C(46)	2013(5)	12172(3)	1559(3)	44(2)
C(27)	14648(5)	3954(4)	2258(4)	58(2)	C(47)	1892(5)	12206(4)	2358(3)	47(2)
C(28)	14065(6)	4797(5)	1710(4)	67(3)	C(48)	1513(4)	11354(4)	2971(3)	46(2)
C(29)	12827(5)	5329(4)	1952(3)	51(2)	C(49)	1215(4)	10469(4)	2777(3)	36(2)
	Chloroform[b]					Chloroform[c]			
C(50)	4969(7)	7507(5)	3340(4)	55(3)	C(50a)	4422(20)	6743(15)	3607(12)	47(5)[d]
Cl(1)	5689(2)	7011(1)	4252(1)	41(1)	Cl(1a)	3091(7)	7673(7)	3220(6)	155(4)
Cl(2)	4964(2)	6517(1)	2842(1)	71(1)	Cl(2a)	5173(6)	6285(4)	2701(5)	84(3)
Cl(3)	3319(2)	8091(2)	3540(1)	66(1)	Cl(3a)	5552(12)	7114(8)	3984(7)	151(6)

[a] One-third trace of orthogonalised U_{ij} tensor [b] Occupancy: 75% [c] Occupancy: 25% [d] Isotropic

Table 22. Comparison of equivalent bond lengths (Å).

Atoms*	Molecule A		Molecule B	
O(10) - C(10)	1.414(5)	1.417(5)	1.422(4)	1.423(5)
C(10) - C(11)	1.511(6)	1.517(6)	1.524(5)	1.523(5)
C(11) - O(11)	1.201(4)	1.199(5)	1.200(4)	1.187(5)
C(11) - O(12)	1.327(5)	1.331(6)	1.329(4)	1.336(5)
O(12) - C(12)	1.447(7)	1.441(6)	1.447(5)	1.442(5)
C(10) - N(10)	1.447(5)	1.435(5)	1.438(5)	1.440(6)
N(10) - C(13)	1.357(5)	1.352(5)	1.364(6)	1.351(5)
C(13) - O(13)	1.227(5)	1.227(4)	1.226(5)	1.226(4)
C(13) - C(14)	1.492(5)	1.488(6)	1.492(6)	1.490(6)
C(14) - C(15)	1.380(6)	1.386(5)	1.382(7)	1.383(5)
C(14) - C(19)	1.382(5)	1.390(6)	1.394(6)	1.395(6)
C(15) - C(16)	1.390(6)	1.385(6)	1.383(7)	1.384(7)
C(16) - C(17)	1.364(6)	1.365(8)	1.384(7)	1.369(7)
C(17) - C(18)	1.370(6)	1.383(8)	1.367(8)	1.380(6)
C(18) - C(19)	1.385(6)	1.393(7)	1.378(6)	1.382(8)

* The four columns refer to bonds between atoms labelled 1X, 2X, 3X and 4X respectively.

Table 23. Comparison of equivalent bond angles (Å).

Atoms*	Molecule A		Molecule B	
C(10) - O(10) - C(20)	112.7(3)		112.5(3)	
O(10) - C(10) - C(11)	108.3(3)	106.8(3)	106.5(3)	107.0(3)
O(10) - C(10) - N(10)	111.8(3)	112.2(3)	111.8(3)	112.3(3)
C(11) - C(10) - N(10)	113.1(3)	110.9(3)	113.0(3)	108.7(3)
C(10) - C(11) - O(11)	124.8(3)	125.9(4)	124.4(3)	125.7(3)
C(10) - C(11) - O(12)	109.5(3)	108.6(3)	110.2(3)	108.3(3)
O(11) - C(11) - O(12)	125.3(4)	125.5(4)	125.1(3)	126.0(3)
C(11) - O(12) - C(12)	117.2(3)	115.6(3)	115.6(3)	115.6(3)
C(10) - N(10) - C(13)	120.1(3)	123.2(3)	120.3(3)	121.8(3)
N(10) - C(13) - O(13)	120.9(3)	121.6(4)	121.5(4)	120.8(4)
N(10) - C(13) - C(14)	116.7(3)	116.5(3)	116.1(4)	117.1(3)
O(13) - C(13) - C(14)	122.4(3)	121.8(3)	122.3(4)	122.1(4)
C(13) - C(14) - C(15)	117.8(3)	123.9(3)	117.3(4)	123.6(4)
C(13) - C(14) - C(19)	123.3(4)	116.6(3)	123.3(4)	117.1(3)
C(15) - C(14) - C(19)	118.9(4)	119.5(4)	117.3(4)	119.2(4)
C(14) - C(15) - C(16)	120.5(4)	120.5(4)	119.8(4)	120.3(4)
C(15) - C(16) - C(17)	119.8(4)	119.9(4)	119.3(4)	120.0(4)
C(16) - C(17) - C(18)	120.5(4)	120.6(5)	120.0(4)	120.5(5)
C(17) - C(18) - C(19)	120.0(4)	119.9(5)	120.2(4)	119.8(5)
C(14) - C(19) - C(18)	120.3(4)	119.6(4)	120.3(5)	120.1(4)

* The four columns refer to angles subtended at atoms labelled 1X, 2X, 3X and 4X respectively.

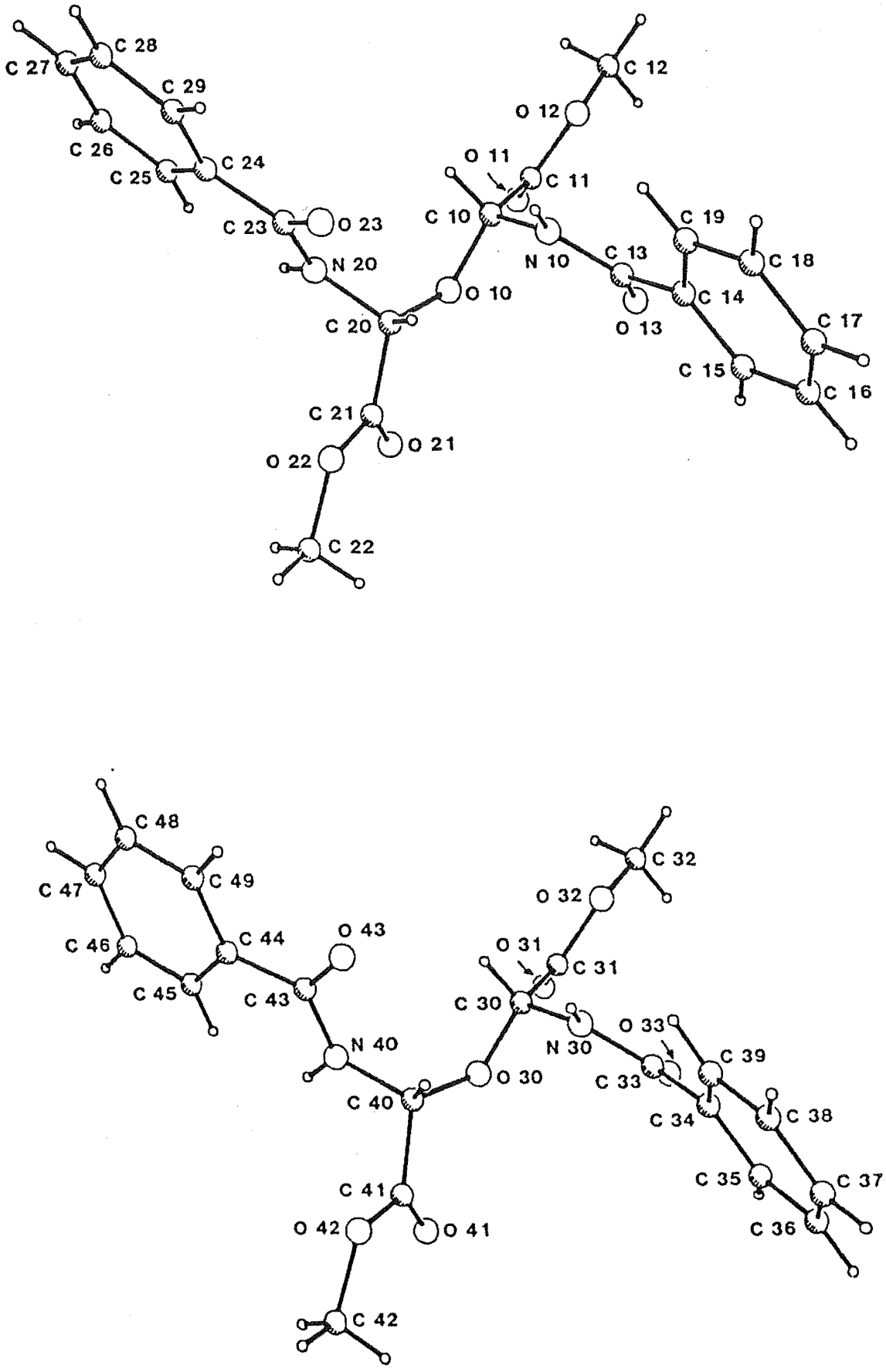


Figure 6. Perspective view and atom labelling of the two independent molecules.

Table 24. Hydrogen bonding parameters.

N(10) - H(10) ..... O(43)*:

O(43)*	=	O(43) [1 + x, y, z]
N-O	=	2.835(5) Å
H-O	=	1.98(4) Å
N-H-O	=	148(3)°

N(20) - H(20) ..... O(21)*:

O(21)*	=	O(21) [1-x, -y, -z]
N-O	=	3.028(5) Å
H-O	=	2.48(5) Å
N-H-O	=	116(4)°

N(30) - H(30) ..... O(23)*:

O(23)*	=	O(23)[x-1, y, z]
N-O	=	2.853(5) Å
H-O	=	2.02(4) Å
N-H-O	=	144(3)°

N(40) - H(40) ..... O(31)*:

O(31)*	=	O(31)[-1-x, 1-y, -1-z]
N-O	=	2.947(5) Å
H-O	=	2.12(4) Å
N-H-O	=	136(3)°

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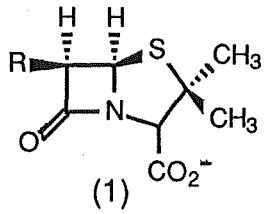

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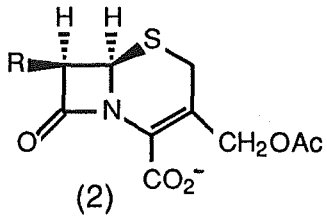

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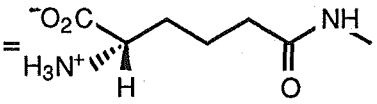
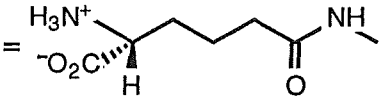
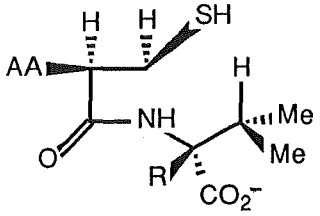
(b) R = 

(c) R = 



(3)

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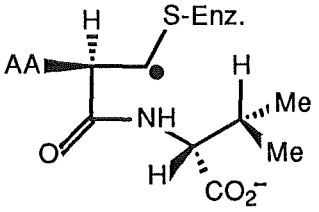

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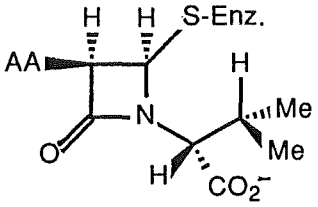

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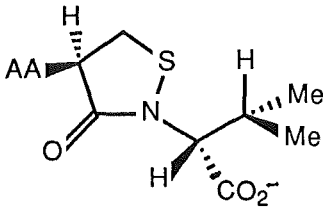

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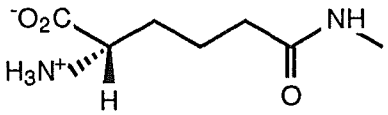
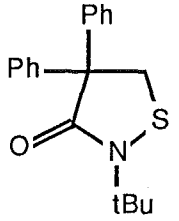
AA = 



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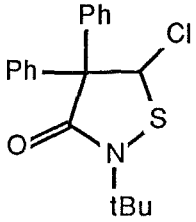
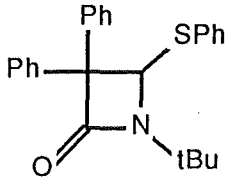
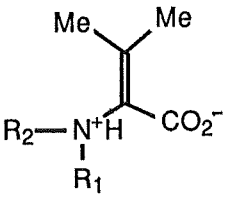


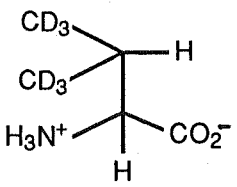
(8)



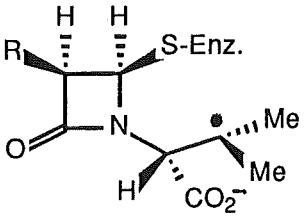
(9)



(10)

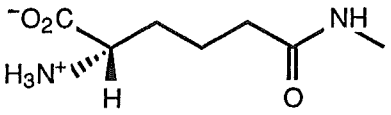


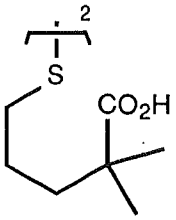
(11)



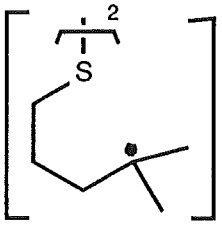
(12)

R =

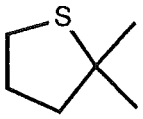




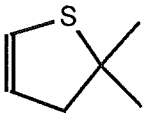
(13)



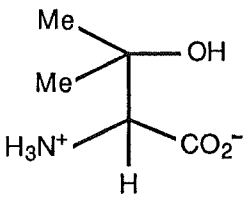
(14)



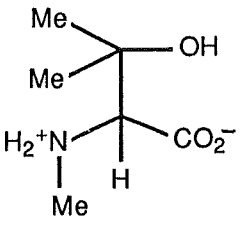
(15)



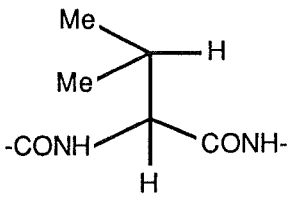
(16)



(17)



(18)



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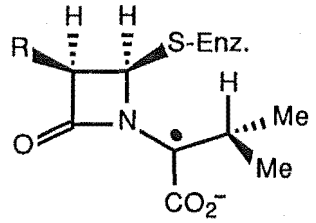

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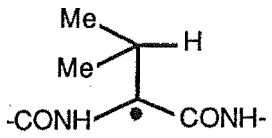
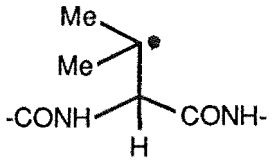
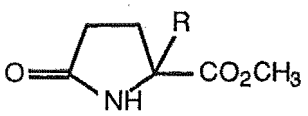


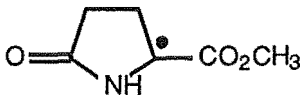
(21)



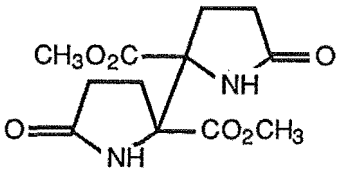
(22)



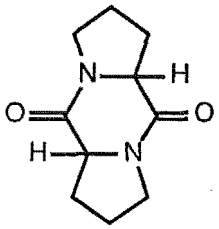
(23)
(a) R = H
(b) R = D



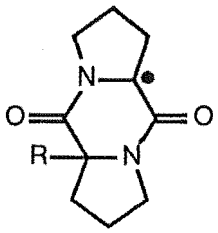
(24)



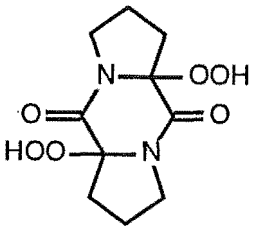
(25)



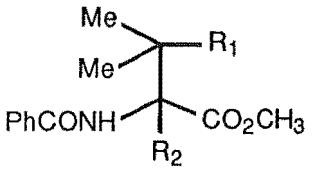
(26)



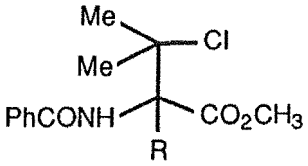
(27)
(a) R = H
(b) R = OOH



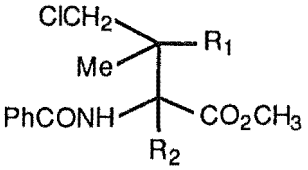
(28)



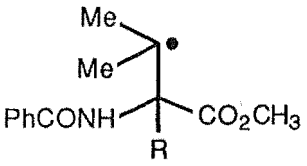
(29)
(a) $R_1 = R_2 = H$
(b) $R_1 = D$, $R_2 = H$
(c) $R_1 = H$, $R_2 = D$



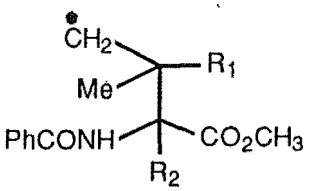
(30)
(a) R = H
(b) R = D



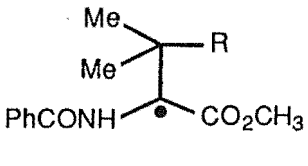
(31)
(a) $R_1 = R_2 = H$
(b) $R_1 = D$, $R_2 = H$
(c) $R_1 = H$, $R_2 = D$



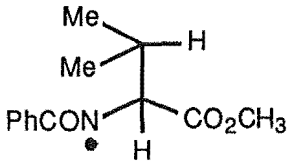
(32)
(a) R = H
(b) R = D



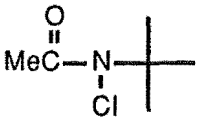
(33)
(a) $R_1 = R_2 = H$
(b) $R_1 = D$, $R_2 = H$
(c) $R_1 = H$, $R_2 = D$



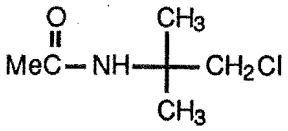
(34)
(a) R = H
(b) R = D



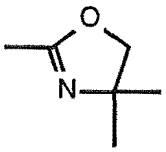
(35)



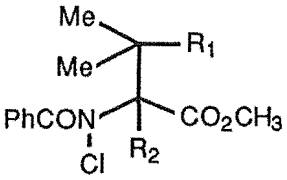
(36)



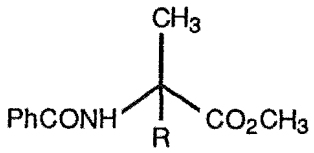
(37)



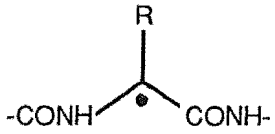
(38)



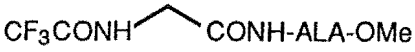
(39)
(a) $R_1 = R_2 = H$
(b) $R_1 = D$, $R_2 = H$
(c) $R_1 = H$, $R_2 = D$



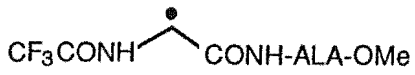
(40)
(a) R = H
(b) R = D



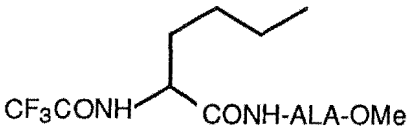
(41)
(a) R = H



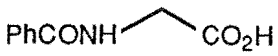
(42)



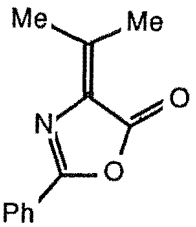
(43)



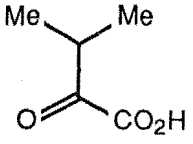
(44)



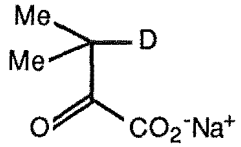
(45)



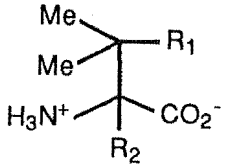
(46)



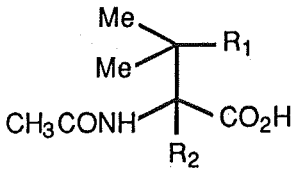
(47)



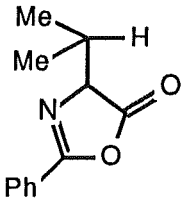
(48)



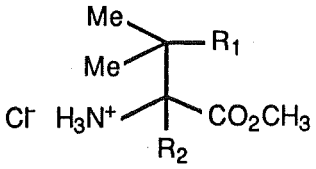
(49)
(a) $R_1 = R_2 = H$
(b) $R_1 = D$, $R_2 = H$
(c) $R_1 = H$, $R_2 = D$



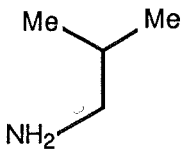
(50)
(a) $R_1 = R_2 = H$
(b) $R_1 = D$, $R_2 = H$
(c) $R_1 = H$, $R_2 = D$



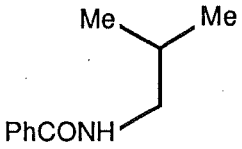
(51)



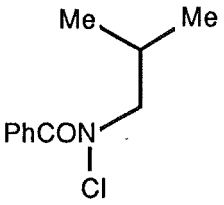
(52)
(a) $R_1 = R_2 = H$
(b) $R_1 = D$, $R_2 = H$
(c) $R_1 = H$, $R_2 = D$



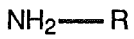
(53)



(54)

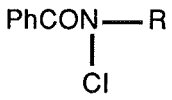


(55)



(56)

(57)
(a) R = t-Bu
(b) R = Ph



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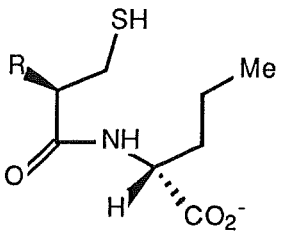
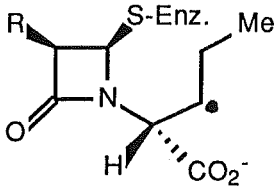


(59)



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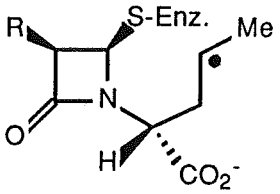
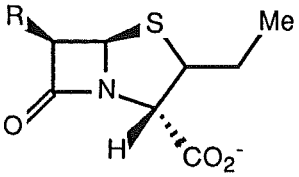


(61)



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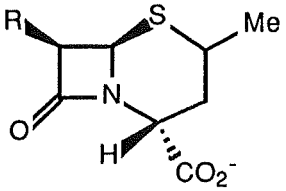
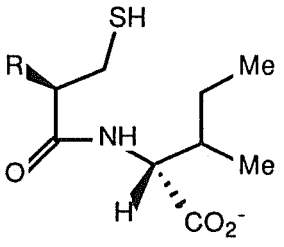


(63)



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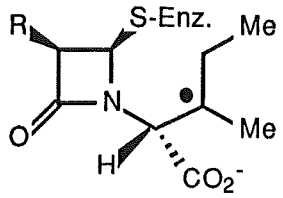

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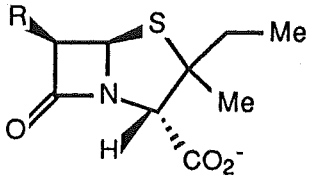

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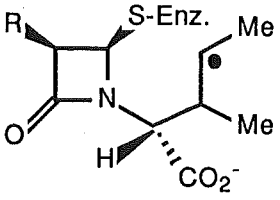

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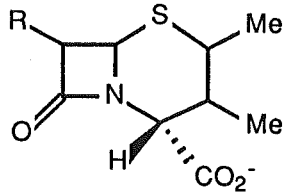

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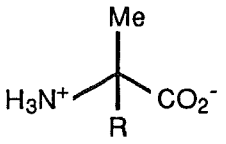
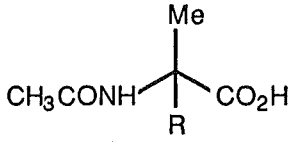
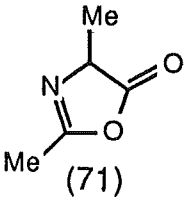


(69)
(a) R = H
(b) R = D



(70)
(a) R = H
(b) R = D



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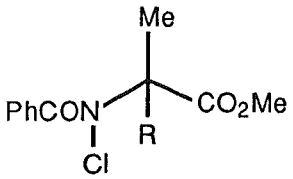
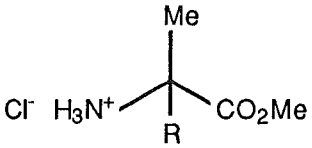


(73)
(a) R = H
(b) R = D



(72)
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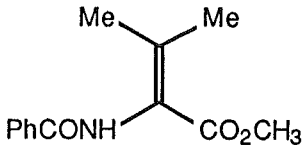
(b) R = D



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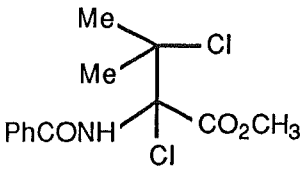

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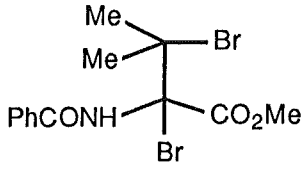

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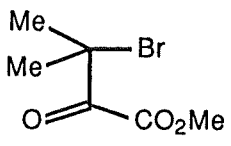
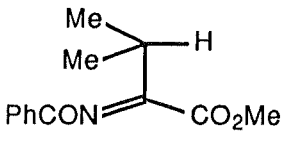
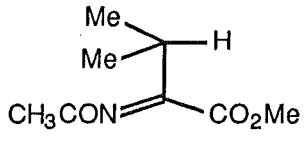


(77)



(78)



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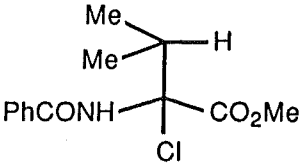

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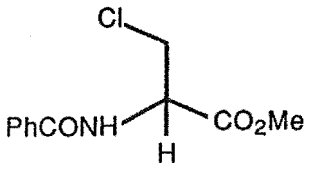

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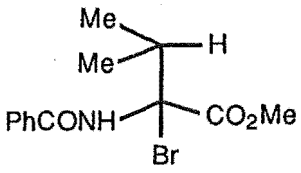
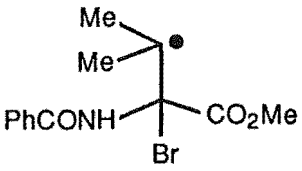
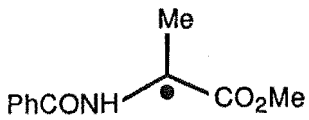


(82)



(83)



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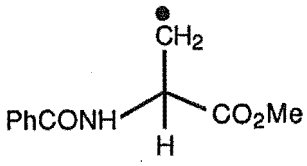

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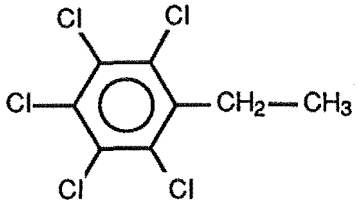
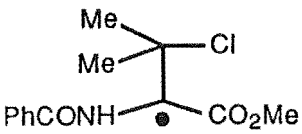
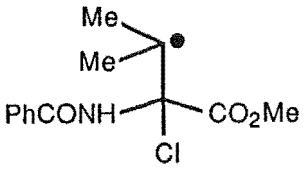


(86)



(87)



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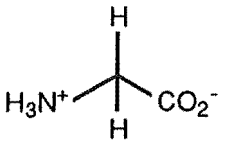
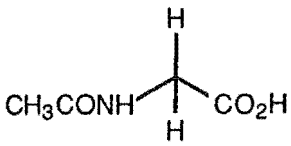


(89)



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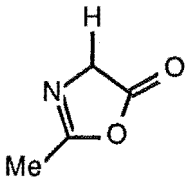
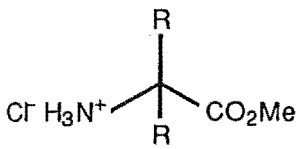
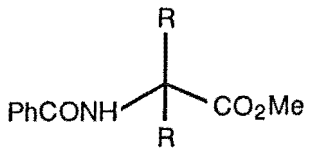


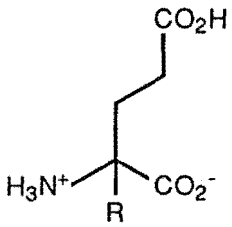
(91)



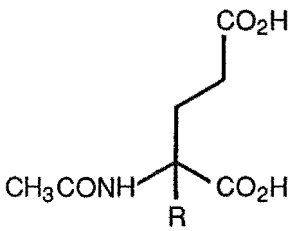
(92)
(a) R = H
(b) R = D



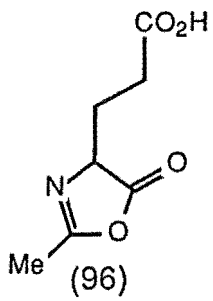
(93)
(a) R = H
(b) R = D



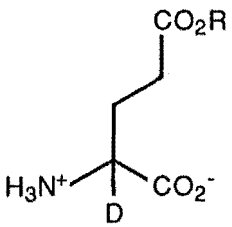
(94)
(a) R = H
(b) R = D



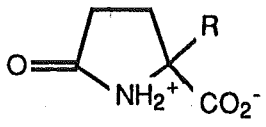
(95)
(a) R = H
(b) R = D



(96)



(97)
(a) R = Et
(b) R = Me



(98)
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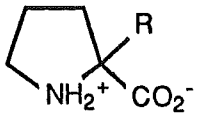
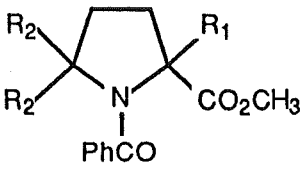
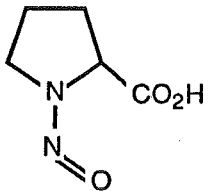
(b) R = D



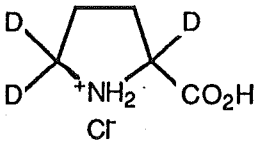
(99)
(a) R = H
(b) R = D



(100)
(a) $R_1 = R_2 = H$
(b) $R_1 = D$, $R_2 = H$
(c) $R_1 = R_2 = D$



(101)



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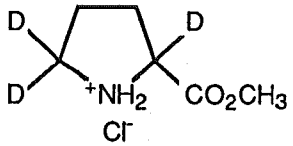
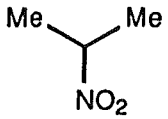
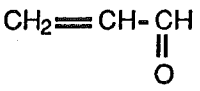


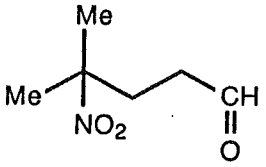
(103)



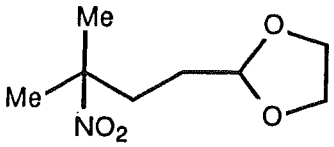
(104)



(105)



(106)



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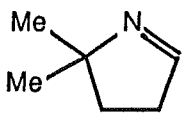
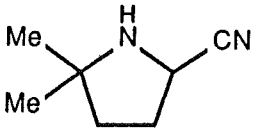
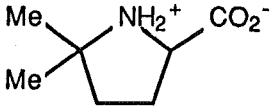


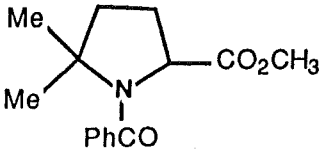
(108)



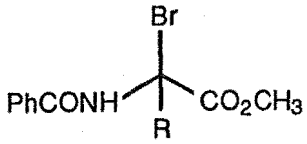
(109)



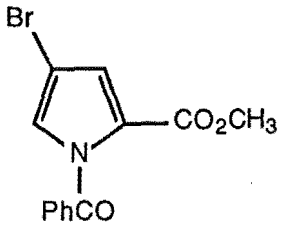
(110)



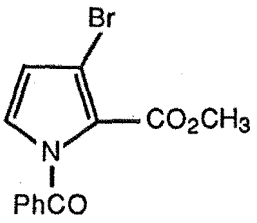
(111)



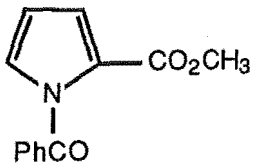
(112)
(a) R = H
(b) R = Br



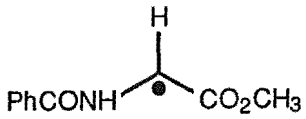
(113)



(114)



(115)



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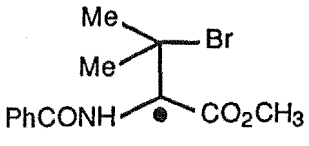
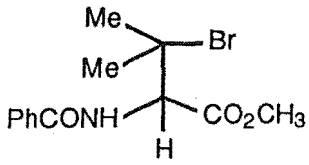
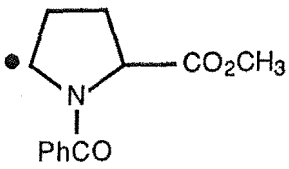


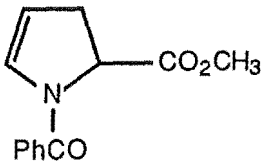
(117)



(118)



(119)



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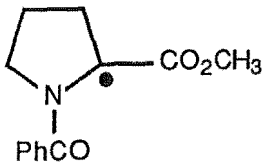
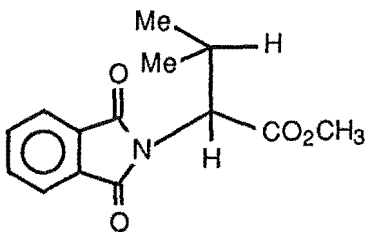
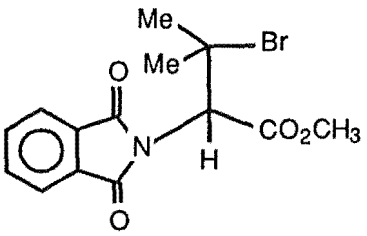


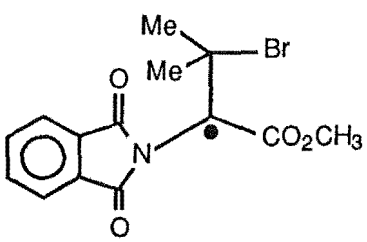
(121)



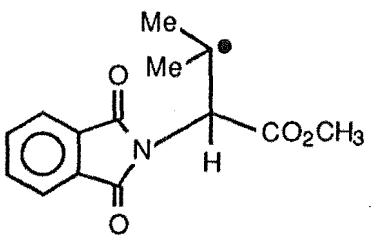
(122)



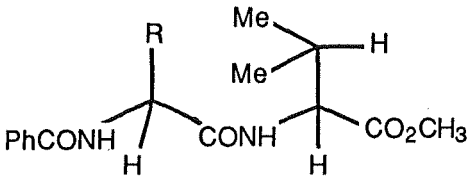
(123)



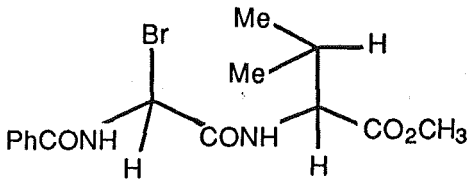
(124)



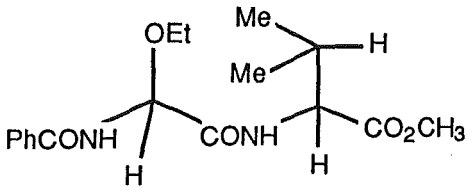
(125)



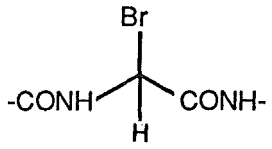
(126)
(a) R = H
(b) R = D



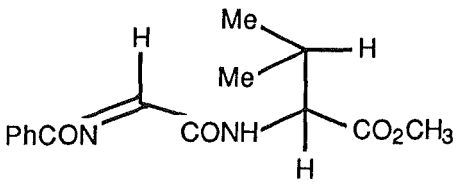
(127)



(128)



(129)



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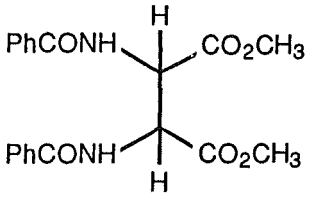
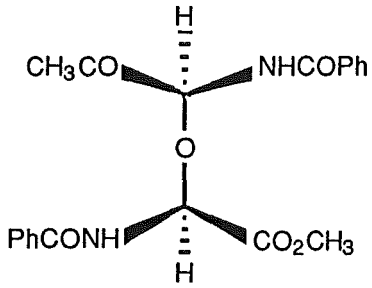
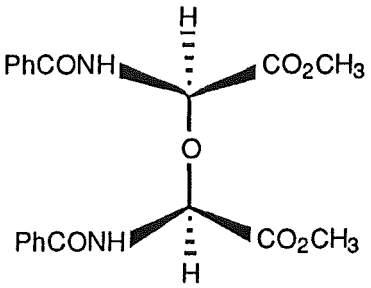


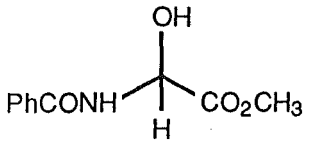
(131)



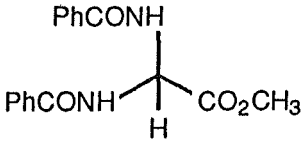
(S,S)-(132a)



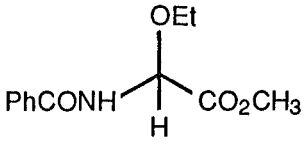
(R,S)-(132b)



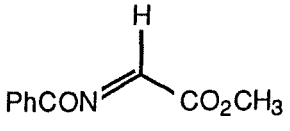
(133)



(134)



(135)



(136)